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Bibliography of Literature Pertaining to the Genus *Selenastrum*

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This report supplements the Selenastrum capricornutum Printz Algal Assay Bottle Test EPA 600/9-78-018

16. ABSTRACT

This bibliography supplements the 1978 Algal Assay protocol by compiling the aveilable literature, published and unpublished, on research that contained algal assays using Selenastrum. The papers cited in this bibliography include studys of the effects of nutrients, toxicants, complex wastes, and specific inorganic and organic compounds upon Selenastrum's growth and physiology. The result is a collection of abstracts, citations, and key words describing 220 papers. This bibliography is designed to be used as a public reference for managers of water quality programs and scientists or educators with interests in research pertaining to Selenastrum and algal assays.

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BIBLIOGRAPHY OF LITERATURE PERTAINING TO THE GENUS *SELENASTRUM*

by

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FOREWORD

Effective regulatory and enforcement actions by the Environmental Protection Agency would be virtually impossible without sound scientific data on pollutants and their impact on environmental stability and human health. Responsibility for building this data base has been assigned to EPA's Office of Research and Development and its 15 major field installations, one of which is the Corvallis Environmental Research Laboratory (CERL).

The primary mission of the Corvallis Laboratory is research on the effects of environmental pollutants on terrestrial, freshwater, and marine ecosystems; the behavior, effects and control of pollutants in lake and stream systems; and the development of predictive models on the movement of pollutants in the biosphere.

This report is a supplement to the 1978 *Selenastrum capricornutum Printz Algal Assay Bottle Test*. It reflects the varied and world-wide application of algal assays in water quality assessment and management.

J. C. McCarty
Acting Director, CERL

PREFACE

The 1976 Experimental Use of Algal Cultures in Limnology Symposium (held in Norway) centered around the use of algae as sensitive indicators to define the stimulatory and/or inhibitory effects of pollutants entering aquatic ecosystems.

The necessity of a standardized test protocol to insure comparative results throughout differing geographic regions and in waters of varying quality was recognized. The Algal Assay Procedure Bottle Test (U.S. EPA, 1971) was cited by Chiaudani and Vighi (1978) as the only algal growth test that had been evaluated and refined enough to be a reliable and reproducible test. This test protocol was endorsed into the 14th edition of Standard Methods for the Examination of Water and Wastewater (APHA, 1975). A revised and updated document, The Selenastrum capricornutum Algal Assay Bottle Test was published by Miller, Greene and Shiroyama in 1978. This document is the foundation of the algal assay growth potential and effluent testing protocols currently in the ballot process for inclusion in the American Society for Testing and Materials Annual Book of ASTM Standards for water. The revised bottle test (1978) new product evaluation section is the basis for a toxicity assay screening protocol which is in the validation process.

Algal assays are a sensitive test for evaluating the effects of heavy metals and for predicting the future effects of toxics in aquatic systems (Chiaudani and Vighi, 1978). Recognition of the application of algal assays and the usefulness of their results for water quality management is increasing throughout the world. The 1978 Algal Assay protocol (Miller, Greene and Shiroyama, 1978) has been distributed throughout the United States and to more than 40 foreign countries.

The purpose of this bibliography is to supplement the 1978 Algal Assay protocol by compiling the available literature, published and unpublished, on research that contained algal assays using Selenastrum. The papers cited in this bibliography include studys of the effects of nutrients, toxicants, complex wastes, and specific inorganic and organic compounds upon Selenastrum's growth and physiology. The result is a collection of abstracts, citations, and keywords describing 220 papers. This bibliography is designed to be used as a public reference for managers of water quality programs and scientists or educators with interests in research pertaining to Selenastrum and algal assays.

A general review of the cited literature indicates that the standard algal assay procedure was followed, but often with modifications to meet the needs of each investigation. In order to compare and utilize the results of similar studies, standardization of the algal assay procedure must be maintained. When deviations from the normal test procedures are deemed necessary, the standard procedure should be performed simultaneously for control purposes. The modified algal assay results can then be compared to those achieved by following the recommended procedures and recognized improvements in methodology can be used to improve the standard procedure. This, hopefully, will be a continuing process. Review of the literature also indicates that a trend has been developing over the years towards a more consistent practice of following standard algal assay procedures such as use of standards: growth media; growth conditions (i.e., light, temperature); inoculum size; and, methods used to measure and assess growth. Utilization of a standardized algal assay procedure, using a selected universal organism, would continue the advancements made towards building a comparable and highly applicable data base for management of water quality.

This bibliography would not have been possible without the generous efforts of those individuals who recognized the requests for literature pertaining to Selenastrum and responded with reprints and/or citations of their research papers. The kindness of the individuals and organizations that granted us the privilege of reproducing copyrighted abstracts and summaries is greatly appreciated. Without these the usefulness of the bibliography would have been greatly reduced. The patience and continued efforts of Zilla Toohill and Bonnie Byron in typing the bibliography are also greatly appreciated.

A standard format was followed for the presentation of the literature. Each paper is presented as: a complete citation with an associated accession number; a list of keywords; and, an abstract or summary provided by the author(s) or the properly acknowledged source. An alphabetized keyword index and author index follows the bibliographic list.

The format for the citations was derived from that followed by the journal Limnology and Oceanography (American Society of Limnology and Oceanography) and the 4th Edition Council of Biology Editors Style Manual (Council of Biology, Editors Inc. 1978). Abbreviations for the journals followed Biosis: List of Serials (BioScience Information Service, 1977). Exceptions to the basic format are:

1) The accession number was designed initially as a four-digit number. As a result of papers being added after the first number assignments and because we wished to maintain alphabetization of authors names, lower case letters were added to the four-digit number, where affected.

2) Citations are provided with brief keyword lists for two papers (0098-b, 0098-c) that were not retrievable. A citation, keyword list and an abstract are provided for paper 0085-a, also not retrievable. Either the translations of the papers' title or source were in error. An extensive library search for these papers proved fruitless, but it was decided to include the citations. Hopefully, someone may recognize and correct the information that has been provided.

3) A few of the papers were published in a foreign language. All are provided with the translated title, source and abstract or summary. The language in which the paper was published is indicated in the citation.

4) Additions to the bibliography were not accepted after 1 April, 1979 so that the project could move toward publication.

5) It is requested that copies of papers using Selenastrum in algal assays that are not included in this bibliography, and any future papers discussing Selenastrum, be forwarded to Joseph C. Greene, Corvallis Environmental Research Laboratory, Corvallis, Oregon 97330, to be included in a future addendum.

REFERENCES

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1975. Standard methods for the examination of water and wastewater, 14th edition. American Public Health Association, Washington, D.C. 1193 p.

BioSciences Information Service. 1977. Biosis: List of serials. BioSciences Information Services, Philadelphia, Pennsylvania. 251 p.

Chiaudani, G., and M. Vighi. 1978. The use of Selenastrum capricornutum batch cultures in toxicity studies. Mitt. Int. Ver. Limnol. 21:316-329.

Council of Biology Editors, Inc. 1978. Council of Biology Editors style manual. A guide for authors, editors, and publishers in the biological sciences. 4th edition. Amer. Inst. Biol. Sci., Arlington, Virginia. 265. p.

Miller, W.E., J.C. Greene, and T. Shiroyama. 1978. The Selenastrum capricornutum Printz Algal Assay Bottle Test: Experimental design, application, and data interpretation protocol. U.S. Environmental Protection Agency, Corvallis, Oregon. EPA-600/9-78-018. 125 p.

U.S. Environmental Protection Agency. 1971. Algal Assay Procedure Bottle Test. U.S. Environmental Protection Agency, Corvallis, Oregon. 82 p.

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1.0 Bibliography

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0001-a

Adam, V. D., D. B. Porcella, and M. L. Cleave. 1978. The biological role of specific compounds in aquatic ecosystems produced by oil shale development, p. 73-74. *In* Utah Water Res. Lab. & Center Water Res. Res., Annual Report UWRL G-78/03.

bottle test, algal assay, oil shale, leachates, lake waters, algistatic, salt loading, stimulation, algae, *Selenastrum capricornutum*, phytoplankton, *Synedra delicatissina*, *Scenedesmus quadricauda*, diatoms.

The development of the soil shale industry will produce large quantities of retorted shale during shale oil production. This retorted shale contains high levels of salt substances and some organic compounds, and water passing through it will leach these salts and possibly the organics into downstream surface and groundwaters. This salt-laden water could have a significant impact on the growth of freshwater algae if it enters the Colorado River system. Since algae are the basis for the food chain in the river, any effect on the algal productivity could affect the food supply for all higher life forms in the river. Since the salts common in the retorted shale are also common to the soil in the region, laboratory experiments that determine the effects of increased salt loading on algae are also applicable in determining the effects of irrigation practices and other water usage which causes evaporative losses in the river system. (Abstract-Author).

0001-b

American type culture collection. 1975. Collections of algae and protozoa - List of strains. ATCC, Rockville, Maryland. 9 p.

algae, cultures, protozoa, *Selenastrum capricornutum* ATCC #22662.

A list of strains of algae and protozoa available and the ATCC classification number.

0001

Aronson, J. G. 1973. The effect of some common insecticides upon C-14 uptake in phytoplankton. M.S. thesis, Univ. Nebraska, Lincoln. 96 p.

carbon-14, insecticides, organochlorine compounds, algae, *Anabaena flos-aquae*, *Microcystis aeruginosa*, *Selenastrum capricornutum* Printz, algal assay.

The acute effect of the twelve organochlorine compounds DDT, DDE, BHC, methoxychlor, lindane, chlordane, aldrin, dieldrin, endrin, toxaphene, endosulfan, and mirex upon the carbon-14 uptake of *in situ* and *in vitro* phytoplankton was determined utilizing the C-14 technique of Steeman-Nielsen. The *in situ* experiments were performed in Pawnee Reservoir, Lancaster County, Nebraska. The *in vitro* experiments were performed using unialgal cultures of *Anabaena flos-aquae* (Lyngb.) DeBrebisson, *Microcystis aeruginosa* Kutzing amend Elenkin *(Anacystis cyanea* Drouet and Daily), and *Selenastrum capricornutum* Printz. Initial concentrations of 1.0ppm, 0.1ppm, 0.01ppm, and 1.0ppb of each compound were used for each series of tests.

In situ C-14 uptake was reduced by all twelve chemicals. DDT, its decomposition product DDE, and a close structural relative, methoxychlor, were most effective in reducing *in situ* C-14 uptake. *In vitro* results were variable. While the C-14 uptake of *Anabaena flos-aquae* cultures was reduced by all twelve chemicals, that of the *Microcystis aeruginosa* cultures was generally enhanced. Cultures of *Selenastrum capricornutum* were relatively unaffected by any of the chemicals. (Abstract-Author).

0002

Bartlett, L., F. W. Rabe, and W. H. Funk. 1974. Effects of copper, zinc, and cadmium on *Selenastrum capricornutum*. Water Res. 8(3):179-185.

zinc, copper, cadmium, algae, *Selenastrum capricornutum*, algicidal, algistatic, bottle test, rivers, algal assay.

The algicidal and algistatic effects of copper, zinc and cadmium on *Selenastrum capricornutum*, a unicellular green algae were analyzed by using a modification of the Algal Assay Procedure Bottle Test.

Algicidal concentrations of copper, zinc and cadmium were 0.30, 0.70, and 0.65 mg l^{-1}. Treatment of *Selenastrum* with various concentrations of the metals resulted in similar growth rates characterized by extended lag growth phases.

Combinations of copper, zinc and cadmium were similar in toxicity to equal concentrations of zinc. Combinations of copper and cadmium resulted in a greater growth rate than equal concentrations of copper suggesting that cadmium inhibits copper toxicity.

Selenastrum was able to exist in waters from the upper South Fork and North Fork of the Coeur d'Alene River where zinc and other metals were in low concentrations. However, the algae was not able to tolerate zinc concentrations greater than 0.5 mg l^{-1} from waters of other parts of the drainage. These observations were consistent with laboratory findings where 0.7 mg l^{-1} zinc was algicidal and 0.1 mg l^{-1} inhibited the growth of Selenastrum. (Abstract-Author).

0003

Behnken, D. W., and D. G. Watts. 1972. Bayesian estimation and design of experiments for growth rates when sampling from the Poisson distribution. Biometrics 28(4):999-1009.

data interpretation, experimental design, Poisson distribution, algae, Selenastrum capricornutum, growth rates, statistical methods, algal assay.

Data on algal growth rates were used to investigate the problem of estimating the growth rate parameter, β, for a process with an expected value λ_i equal to $\alpha e^{\beta t_i}$. This equation produces data from a Poisson distribution P of lambda λ_i at time t, (i equals 1,2,.....n). The posterior distribution for β is derived, and an approach to designing experiments in this situation is suggested. (Abstract-Little-Battelle WRSIC).

0003-a

Bentley, R. E., J. W. Dean, S. J. Ells, T. A. Hollister, G. A. LeBlanc, S. Sauter, and B. H. Sleight. 1977. Laboratory evaluation of the toxicity of cyclotrimethylene trinitramine (RDX) to aquatic organisms. Final report, E G & G Bionomics, Wareham, Mass. DAMD-17-74-C-4101. 86 p.

toxicity, algistatic, cyclotrimethylene trinitramine, RDX, algal assay, bottle test, chlorophyll a, algae, Microcystis aeruginosa, Selenastrum capricornutum, Anabaena flos-aquae, diatoms, Navicula pelliculosa, fish, water quality.

The toxicity of nitroglycerine to a wide variety of aquatic organisms representing several different trophic levels in aquatic ecosystems was studied. Results of static acute toxicity tests indicate that the acute LC_{50} values are greater than 3 mg/l RDX. There was an apparent lack of bioaccumulation in edible or nonedible tissues or organs in all species tested. Effects were observed on growth at 5.8 mg/l RDX during egg and fry studies, on survival at 4.9-63 mg/l during chronic exposure of fathead minnows, and on number of young produced per parthenogenetic female at concentrations $\geq$ 4.8 mg/l. Applying an application factor of 0.1 to the lower limit of observed acute toxicity values, (3.6 mg/l, 96-hour LC50 for bluegill at pH 6.0, 20°C) a water quality criterion of 0.35 mg/l RDX is proposed for the protection of freshwater aquatic life with an adequate margin of safety. (Abstract-Author).

0003-b

Bentley, R. E., G. A. LeBlanc, T. A. Hollister, and B. H. Sleight III. 1977. Acute toxicity of 1,3,5,7-tetranitro-octahydro-1,3,5,7-tetrazocine (HMX) to aquatic organisms. Final report, E G & G, Bionomics, Wareham, Mass., DAMD-17-74-C-4101. 23 p.

toxicity, HMX, 1,3,5,7-tetranitro-octahydro-1,3,5,7-tetrazocine, water quality, phytoplankton, fish, algae, Microcystis aeruginosa, Anabaena flos-aquae, Selenastrum capricornutum, diatoms, Navicula pelliculosa, algal assay, bottle test.

The acute toxicity of 1,3,5,7-tetranitro-octahydro-1,3,5,7-tetrazocine (HMX) was studied utilizing aquatic organisms representing several different trophic levels in aquatic ecosystems. Generally, no adverse effects of exposure to 32 mg/l HMX were observed among any of the algae, fish, or invertebrate species tested. The 7-day old fry of the fathead minnow were the only life stage or species acutely affected. Based on an application factor of 0.05 and a 96-hour LC_{50} for the most sensitive aquatic organism (7-day old fry of the fathead minnow) tested (15 mg/l), a water quality criterion of 0.75 mg/l is proposed for the protection of freshwater aquatic life with an adequate margin of safety. (Abstract-Author).

0003-c

Bentley, R. E., J. W. Dean, S. J. Ells, G. A. LeBlanc, S. Sauter, K. S. Buxton, and B. H. Sleight, III. 1978. Laboratory evaluation of the toxicity of nitroglycerine to aquatic organisms. Final report. E G & G, Bionomics, Wareham, Mass. DAMD-17-74-C-4101. 82 p.

toxicity, nitroglycerine, water quality, algistatic, algal assay, bottle test, chlorophyll a, algae, Microcystis aeruginosa, Anabaena flos-aquae, Selenastrum capricornutum, diatoms, Navicula pelliculosa.

The toxicity of nitroglycerine to a wide variety of aquatic organisms representing several different trophic levels in aquatic ecosystems was studied. The results of static acute toxicity tests indicate that the nominal acute LC_{50} values are generally greater than 1 mg/l nitroglycerine. A pilot bioaccumulation study with C-14-nitroglycerine showed that there was no appreciable accumulation of C-14-nitroglycerine in the edible tissues of four fish species. The estimated application factor for fathead minnow over one complete life cycle is >0.01<0.02 and >0.03<0.07 for eggs and fry of the fathead minnow and channel catfish, >0.2<0.4 for daphnids and >0.3<0.6 for midges. Applying the lower limit of all estimates of the application factor (0.03) to the lower limit of the observed acute toxicity values for freshwater organisms (1.28 mg/l, 96-hour LC_{50} for bluegill at pH 6.0), we propose a water quality criterion of 0.01 mg/l nitroglycerine. (Abstract-Author).

0003-d

Bentley, R. E., J. W. Dean, T. A. Hollister, G. A. LeBlanc, S. Sauter, B. H. Sleight III, and W. G. Wilson. 1978. Laboratory evaluation of the toxicity of elemental phosphorus (P_4) to aquatic organisms. Final Report, E G & G, Bionomics, Wareham, Mass. DAMD-17-74-C-4101. 92 p.

toxicity, phosphorus, algistatic, algal assay, bottle test, chlorophyll *a*, algae, *Microcystis aeruginosa*, *Anabaena flos-aquae*, *Selenastrum capricornutum* diatoms.

The toxicity of elemental phosphorus to a wide variety of aquatic organisms was studied. Results of static acute toxicity tests indicate that the acute LC_{50} values varied over several orders of magnitude with an observed range of 6 - >560 µg/l of elemental phosphorus. Chronic exposure of fathead minnows for 150 days to concentrations ranging from 1.5 - 3.4 µg/l significantly reduced growth. In addition, survival was affected in concentrations ≥1.5 µg/l, while eggs spawned by fish exposed to the low concentrations of 0.71 and 0.40 µg/l failed to hatch when incubated. Although bioaccumulation of elemental phosphorus was low (*ca* 200X), the cumulative mortality pattern observed suggests continuing damage to target organs. Based on the available data and the use of a safety factor of 0.1, an ambient water quality criterion for elemental phosphorus of 0.04 µg/l is recommended (Abstract-Author).

0003-e

Bilcea, R. 1975. Contributions to the knowledge of the influence of nutrition on the growth and development of the alga Selenastrum gracile Reinch. Rev. Romaine de Biol. 20(3):185-191.

algal assay, algae, N:P ratio, Selenastrum gracile Reinsch, soil extraction.

The physiological characters of S. gracile grown in various nutritive media with a small soil extract supplement in various concentrations were studied. The alga thrived on concentrated nutritive media with a N:P ratio of 5.5:1. The soil extract at a concentration of 1% stimulated the multiplication of the alga in all the nutritive media. (Abstract-Biological Abstracts, Inc.).

0004

Bishop, N. I., M. Frick, and L. W. Jones. 1977. Photohydrogen production in green algae: Water serves as the primary substrate for hydrogen and oxygen production, p. 3-22. In A. Mitsui, S. Miyachi, A. San Pietro, and S. Tamura [eds.], Biological solar energy conversion, Academic Press, New York.

DCMU, CCCP, photohydrogen, algae, Selenastrum sp., Ankistrodesmus falcatus, Ankistrodesmus braunii, Scenedesmus, Chlorella fusca, Chlorella pyrenoidosa (211-8k), Chlorella kesslerii, Kirchneriella lunaris, Coelastrum proboscideum, Ulothrix acuminata, Scenedesmus obliquus, algal assay.

Photohydrogen evolution was surveyed in a number of species of algae. The following ones had rates of H_2 evolution comparable to Scenedesmus obliquus: Selenastrum sp., Ankistrodesmus falcatus, Ankistrodesmus braunii, Scenedesmus sp., Kirchneriella lunaris, Coelastrum proboscideum, Ulothrix acuminata, Chlorella fusca, Chlorella pyrenoidosa (211-8K), and Chlorella kesslerii. Maximum rates for autotrophic and heterotrophic: Scenedesmus approached 2 and 10 µl H_2/µl cells/hr respectively, 50-90% inhibition of H_2 production by DCMU was uniformily observed in all of the above species and in manganese deficient Scenedesmus.

Re-examination of photohydrogen production by a series of Scenedesmus mutants blocked in PS-II revealed a nearly complete loss of photohydrogen production. CCCP stimulated the initial low rates but only to about 10% of wild type. We conclude that hydrogen evolution in the species examined requires both photosystems for maximal activity; whether water photolysis is essential for this reaction remains an open question. (Abstract-Author).

0005

Bowman, R. H. 1974. The control of pH by carbon dioxide, TRIS (Hydroxymethyl aminomethane), and other buffers on the Algal Assay Bottle Test. M.S. thesis, Utah State Univ., Logan. 100 p.

algal assay, bottle test, pH, carbon dioxide, TRIS, buffers, algae, *Selenastrum capricornutum*, reservoirs, water hardness.

The purpose of this paper is to discuss the development of a convenient method for using the Environmental Protection Agency's Algal Assay Procedure Bottle Test in hard waters and to demonstrate the usefulness of the technique in assays of Hyrum Reservoir water samples.

This thesis also deals with the problem of using the bottle test in hard water. This problem is that when the pH rises above 9.0, a chemical precipitation is observed on the bottom of the flasks and all algal growth stops. The first step in developing a convenient method for using the assay in hard water was to determine a pH range at which the test alga (*Selenastrum capricornutum*) would grow but precipitation would not occur. Once the pH range was determined, it was necessary to develop a suitable method for controlling the pH in that range.

Two methods for controlling pH were evaluated in this experiment: Tris (hydroxymethyl aminomethane) buffer and CO_2 addition. Other buffers were evaluated in terms of pH control within a specific range, availability and cost, ease of addition and effect of buffer on algal growth. (Abstract-Author).

0006

Brezonik, P. L., F. X. Browne, and J. L. Fox. 1975. Application of ATP to plankton biomass and bioassay studies. Water Res. 9(2):155-162.

ATP, chlorophyll *a*, algal assay, DNA, luminescence, algae, *Anabaena flos-aquae*, *Microcystis aeruginosa*, *Selenastrum capricornutum*, phytoplankton, *Chlorella* sp.

The use of adenosine triphosphate (ATP) analysis for freshwater algal and plankton populations was evaluated as a measure of biomass and as a bioassay response parameter. ATP analysis was performed using the firefly luminescence procedure. In short term laboratory studies, ATP levels in cultured algae and lake plankton correlate well with other standard biomass parameters, including chlorophyll a and dry weight. Algal ATP responded rapidly to mercury addition and pH changes, indicating its usefulness as a measurement of toxicity. The rapid response of ATP following nutrient additions to starved algal cultures suggests ATP may be useful as a tool in nutrient bioassay studies. (Abstract-Author).

0007

Brown, E. J., and R. F. Harris. 1978. Kinetics of algal transient phosphate uptake and the cell quota concept. Limnol. Oceanogr. 23(1):35-40.

phosphate, phosphorus, limiting nutrients, uptake, cell quota, growth kinetics, bottle test, algae, Selenastrum capricornutum, Nostoc sp., algal assay.

Dissolved inorganic orthophosphate (P_1) uptake rates (v) were determined as a function of total cell phosphorus content (Q) in P_1 perturbed axenic batch cultures of Selenastrum capricornutum and Nostoc sp. The results showed an inverse relationship between v and Q for both organisms. These findings are used to relate the cell quota concept of nutrient limited, steady state microbial growth kinetics to nutrient uptake kinetics observed in perturbed batch cultures. (Abstract-Author).

0008

Brown, E. J., R. F. Harris, and J. K. Koonce. 1978. Kinetics of phosphate uptake and aquatic microorganisms: Deviations from a simple Michaelis-Menten equation. Limnol. Oceanogr. 23(1):26-34.

kinetics, phosphate, ^{32}P, uptake, Michaelis-Menten equation, bottle test, algae, Selenastrum capricornutum, phytoplankton, lake waters, algal assay.

The uptake kinetics of dissolved inorganic phosphate ($^{31}P_1$) by lake water microorganisms and a green alga (Selenastrum capricornutum) were determined as a function of uptake rates of radiophosphate ($^{32}P_1$) at added $^{31}P_1$ levels >0. The data did not follow a simple Michaelis-Menten equation. (Abstract-Author).

0008-a

Brown, E. J., and D. K. Button. 1979. Phosphate-limited growth kinetics of *Selenastrum capricornutum* (Chlorophyceae). J. Phycol. In press.

phosphate, limiting nutrients, kinetics, growth rates, continuous flow, nutrients, algal assay, algae, *Selenastrum capricornutum* Printz.

Most theoretical studies of phytoplankton growth in aquatic environments assume that relative nutrient utilization abilities regulate species composition. The steady-state phosphate-limited growth kinetics of *Selenastrum capricornutum* Printz were examined using continuous cultures to characterize the green alga's ability to compete for orthophosphate (Pi) when Pi limits growth. The maximal specific growth rate for *Selenastrum* at 20°C was 1.20 (day)$^{-1}$, and the concentration where half maximal growth rate occurs was 40 nanomolar Pi. There was an apparent threshold of 10 nanomolar Pi. Cell yields varied inversely with growth rate; thus ability to utilize Pi could not be characterized in terms of the Monod half-saturation constant and maximal growth rates. Instead, we computed the Pi affinity from steady-state flux vs. external Pi concentrations. This affinity was 2.8 liter (milligrams dry weight-day)$^{-1}$ for *Selenastrum*. Kinetic evidence from this study suggests that *Selenastrum* will not be growth competitive with some other common aquatic heterotrophs and autotrophs when Pi limits microbial growth in lakes. (Abstract-Author).

0009

Cain, J. R., and F. R. Trainor. 1973. A bioassay compromise. Phycologia 12(3/4):227-232.

algal assay, water quality, nutrients, growth rates, bioindicators, rivers, wastewater, algae, *Selenastrum capricornutum*.

Selenastrum capricornutum was used as a test organism for assaying the nutrient condition of natural waters. Using an actively growing culture, inoculated, sterile-filtered water was incubated at 22° C in continuous fluorescent illumination. Organisms were grown in tubes on a roller tube rotator and transferred daily, re-establishing a population of 4 x 10^5 cells each day. Averages of 5-day runs showed no evidence for nutrient carry-over when 1 + doublings/day were recorded; carry-over usually was not apparent on days 3-5 when nutrient poor waters were examined.

We examined sites above and below a sewage treatment plant, in different rivers and during fall, winter and spring. The assay, as measured in doublings of Selenastrum/day, shows clear differences in the carrying capacity of the rivers at these sites. (Abstract Only-Author).

0009-a

Camp, F. A., J. M. Dolan III, and A. C. Hendricks. 1974. Algal bioassay studies of the effects of bio-degradation on the toxicity of a non-ionic surfactant to Selenastrum capricornutum, Chlorophyceae. ASB Bull. 21(2):45.

wastewater, biodegradation, algal assay, toxicity, algae, Selenastrum capricornutum, bottle test.

A mixture of a nonionic surfactant and synthetic sewage was introduced into bench scale activated sludge units. Retention times of 4 and 12 hours were used to vary the efficiency of biodegradation of the surfactant. Both control and experimental units were operated for each retention time. E.P.A. algal bioassay techniques were used to evaluate the toxicity of the effluents to the test organism Selenastrum capricornutum. Growth was monitored regularly by reading absorbance with a double-beam spectophotometer. Results were statistically significant (a=0.05) in showing that experimental effluents supported essentially the same levels of growth as the control effluents. This was found for both the 4 hour and 12 hour effluents, i.e. no toxic effect was observed. (Abstract Only-Author).

0010

Chiaudani, G., and M. Vighi. 1974. The N:P ratio and tests with Selenastrum to predict eutrophication in lakes. Water Res. 8(12):1063-1069.

nitrate, phosphate, algae, Selenastrum capricornutum, eutrophication, lake waters, limiting nutrients, N:P ratios, algal assay.

During the spring overturn, at the time of maximum potential phytoplankton growth, 26 Italian lakes were sampled to carry out enrichment experiments in which single and mixed treatments of nitrate and phosphate were applied to laboratory cultures of Selenastrum capricornutum. The method, in conjunction with chemical analysis, can be used to classify the lakes and to evaluate the limiting values of each nutrient. It was possible to correlate the phosphorus concentration to the maximal primary production and obtain threshold levels for phosphorus in predicting eutrophication trends in lakes. (Abstract-Author).

0011

Chiaudani, G., and M. Vighi. 1975. Dynamic of nutrient limitation in six small lakes. Verh. Int. Ver. Limnol. 19(2):1319-1324.

bottle test, algal assay, algae, Selenastrum capricornutum, limiting nutrients, lake waters, eutrophication, phosphorus, nitrogen.

Laboratory bioassays of samples which utilized Selenastrum capricornutum (Printz) as the test organism, were carried out monthly in 1972-73 on six small subalpine lakes (the Brianza Lakes) in northern Italy near the southern part of Lake Como. The bioassays were aimed at establishing experimentally which of the essential mineral elements must be removed to restrain or cut down excessive algal growth in lake waters. Measurements were made of orthophosphate phosphorus, total soluble phosphorus, ammonia nitrogen, total inorganic carbon (computed from total alkalinity, acidity and temperature) and three measured nitrogen oxidation forms. Morphological characteristics were examined in the lakes, and a stepwise multiple regression analysis was run to evaluate the statistical relationship between S. capricornutum growth as a dependent variable and different chemical parameters as independent variables. Three conclusions were drawn from this analysis: (1) The Molybdenum Blue Method, as confirmed by biological assays, measures the phosphorus available for algal growth; (2) Orthophosphate phosphorus appears to be the key factor for phytoplankton growth in all six lakes, followed by nitrogen as the factor of second-ranked importance and as verified by the stepwise multiple regression analysis; (3) The evaluation of the nitrogen:phosphorus ratio in lake waters during winter overturn can reasonably permit forecasts of the dynamics of nutrient limitation all through the year. (Abstract-Harris-Wisconsin WRSIC).

0012

Chiaudani, G., and M. Vighi. 1976. Comparison of different techniques for detecting limiting or surplus nitrogen in batch cultures of Selenastrum capricornutum. Water Res. 10(8):725-729.

nitrogen, limiting nutrients, bottle test, N:P ratios, algae, Selenastrum capricornutum, algal assay.

Several metabolic responses of Selenastrum capricornutum grown at different initial N:P ratios were studied in order to determine the degree of nitrogen deficiency when alga enters and leaves nitrogen limitation in batch cultures. The results show a good agreement among the tests and good sensitivity and accuracy for all the used techniques. (Abstract-Author).

0013

Chiaudani, G., and M. Vighi. 1978. The use of Selenastrum capricornutum batch cultures in toxicity studies. Mitt. Int. Ver. Limnol. 21:316-329.

bottle test, toxicity, heavy metals, copper, zinc, cadmium, nickel, chromium, cobalt, lake waters, algae, Selenastrum capricornutum, algal assay.

Selenastrum capricornutum batch cultures are extensively applied to the assessment of algal growth potential and to identify limiting factors.

In the present paper, some results of the use of a standard assay procedure for the identification of toxic effects in natural waters, are presented. The presence of toxic effect is evaluated on the basis of the comparison between observed growth and the growth predicted from the concentration of the limiting nutrient.

Also, some data relative to the toxicity of selected heavy metals as EC_{50} 96-hr on Selenastrum capricornutum grown in AAP completely inorganic medium and in medium with a chelating agent are given.

Finally, with the aim to set up water quality criteria for phytoplankton some results are given relative to the effects of some heavy metals added to natural waters taken from largely different environments. (Abstract-Author).

0013-a

Chillingsworth, M. B. 1974. The toxicity of aminoanthraquinone dyes to fish and algae. Chap. 5. In Dyes in the environment - Reports on selected dyes and their effects, v. II, Amer. Dye Manuf. Inst., Inc., New York.

dyes, fish, Pimephales promelas, algal assay, bottle test, algae, Selenastrum capricornutum, toxicity.

Twelve aminoanthraquinone dyes--Disperse Violet 1, Disperse Blue 3, Solvent Blue II, Solvent Blue 14, Solvent Green 3, Acid Green 25, Acid Violet 34, Acid Blue 25, Disperse Blue 7, Acid Blue 45, Basic Blue 21, and Disperse Red 60--and three anthraquinone compounds--anthraquinone, sodium anthraquinone beta sulfonates, and 1-anthraquinone sulfanilic acid sodium salt--were studied for their toxicity to the fathead minnow, Pimephales promelas, using the acute static bioassay procedure and to the green algae, Selenastrum capricornutum, using the algal assay bottle test. The anthraquinone compounds exhibited no toxic effects to the fish in 96 hours at concentrations of 1 mg/l and 10 mg/l. Symmetrical dyes, Acid Blue 45 and Acid Violet 34, were also nontoxic. The strongly cationic dye, Basic Blue 21, is highly toxic to both fish and algae. The remainder of the dyes showed varying degrees of toxicity related to the degree of ionization. Within this group the pattern of response by fish and algae differs, possibly indicating different toxic mechanisms. Reax 83-A, used to disperse the solvent and disperse dyes tested, or impurities in the dyes may contribute to the toxicity observed. Further investigation of anthraquinone dyes is recommended to elucidate the toxic modes and to enable better predictions of toxicity. (Abstract-Author).

0013-b

Christensen, E. R., and J. Scherfig. 1979. Effects of manganese, copper and lead on Selenastrum capricornutum and Chlorella stigmatophora. Water Res. 13(1):79-92.

algal assay, algae, bottle test, Chlorella stigmatophora, copper, inhibition, lead, manganese, media, Selenastrum capricornutum, toxicity, wastewater, heavy metals.

Algal assays were conducted to observe the responses of Selenastrum capricornutum and Chlorella stigmatophora to the metals manganese, copper and lead added singly or in combination to both artificial media and natural waters. A 50% reduction in the total algal cell volume of Selenastrum in standard algal medium (SAAM) occurred by the addition of either 3.1 mg manganese l^{-1}, 85 μg copper l^{-1}, or 140 μ lead l^{-1}. For Chlorella grown in 28 ppt artificial seawater plus full SAAM nutrients, the corresponding numbers were 50 mg manganese l^{-1}, 70 μg copper l^{-1}, or 700 μg lead l^{-1}. Of the metals tested, only lead was found in higher concentrations in urban and agricultural runoff than at least one of these levels.

Theoretical considerations show that a product model is a more reasonable reference for no-interaction than an additive model for experiments in which toxic metals are added in combination. Combination experiments, in which any combination of the three metals act at the same time indicate (1) synergism between manganese and copper (2) antagonism between manganese and lead and (3) antagonism between copper and lead. As to the manganese-lead interaction, it is shown that the lead-induced inhibition of cell division is offset, partially for _Selenastrum_ and completely for _Chlorella_ by the addition of manganese. (Abstract-Author).

0014

Claesson, A. 1973. Algal assay procedure: Minitest with lake water (preliminary report), p. 35-40. _In_ Algal assays in water pollution research, Proc. Nordic Symp., 25-26 October 1972, Oslo, Norway. NORDFORSK, Secretariat of Environmental Sciences.

lake waters, Minitest, algae, _Selenastrum capricornutum_, _Ankistrodesmus falcatus_, _Chlorella homosphaera_, _Euglena gracilis_, _Scenedesmus quadricauda_, AGP, limiting nutrients, algal assay, bottle test.

The report describes the use of the Minitest (National Swedish Environment Protection Board Algal Assay) to test the growth potential of lake waters and to study the limiting nutrients. The algal species used was _Selenastrum capricornutum_. Results are briefly discussed and growth curves are provided. The author concludes that the algal assay procedure can be successfully conducted with small volumes of test waters, thus the name "Minitest". (Abstract-Leischman-EPA).

0015

Claesson, A. 1975. Algal assay procedures at some Nordic laboratories - a comparison. Vatten 31(4):333-338.

bottle test, algae, _Selenastrum capricornutum_, _Chlorella_ sp., _Chlorella homosphaera_, _Phaeodactylum tricornutum_, _Ankistrodesmus falcatus_, _Oscillatoria agardii_, AGP, limiting nutrient, algal assay, diatoms.

A comparison between the different algal assay procedures used at some Nordic laboratories gave the following results:

1. A comparison between the precision of the electronic particle counters indicated the necessity of corrections. The following intercalibrations then gave good agreements between the countings.
2. The maximal biomass produced varied a great deal between the laboratories. The same experience has been obtained from similar American investigations.
3. The different laboratories determined with good agreement P or N as the most limiting nutrients.
4. The growth curves for Selenastrum in standard medium showed good agreement between three of four participating laboratories when maximal yield (measured as cell volume) was determined, but the algal cells were of different size (mean cell volume). (Summary-Author).

0016

Claesson, A. 1978. Research on recovery of polluted lakes: Algal growth potential and the availability of limiting nutrients. Abstr. Uppsala Diss, ACTA Univ. Upsaliensis No. 461, Uppsala, Sweden. 27 p.

Minitest, lake waters, AGP, algal assay, limiting nutrients, algae, Selenastrum capricornutum, wastewater, N:P ratios.

In order to elucidate the effects of advanced wastewater treatment for P removal and sewage diversion, the National Swedish Environment Protection Board started a long-term program in 1972 for analyzing the conditions in a number of wastewater receiving lakes. This thesis summarizes some algal assay results from some of these polluted lakes mainly concerning algal growth potential (AGP), growth-limiting nutrients and availability of algal nutrients. As a result of the present investigation a few general remarks can be made.

- In lake water a total-N: total-P (TN:TP) ratio of $\geq$ 17 and an inorganic-N: inorganic-P (IN:IP) ratio of $\geq$ 12 can be used as critical values for P-limited Selenastrum growth and for N-limited growth a TN:TP ratio of $\leq$ 10 and an IN:IP ratio of $\leq$ 5. These values are suggested for use only when no algal assay data are available.
- A decrease in the nutrient content of a lakewater can result in decreased lake chlorophyll a content or in decreased AGP or in both simultaneously.

- The potential algal biomass should be considered when describing the trophic status of a water body e.g. as "total chlorophyll a".
- The degree of nutrient availability for algal growth of a water sample can be estimated by algal assays.
- The amount of available algal nutrients ought to be considered in nutrient budget calculations. (Abstract-Author).

0016-a

Claesson, A. 1978. Variation in cell composition and utilization of N and P for growth of Selenastrum capricornutum. No. II, Publ. in Abstr. Uppsala Diss. ACTA Univ. Upsaliensis No. 461, Uppsala, Sweden. 20 p.

nutrients, nitrogen, phosphorus, chlorophyll a, growth kinetics, N:P ratio, algal assay, algae, Selenastrum capricornutum.

Yield coefficients (mm^3 cell volume/mg nutrient element) for the green alga Selenastrum capricornutum were determined with NO_3-N ($Y_N = 134$), NH_4-N ($Y_N = 111$) and PO_4-P ($Y_P = 2573$).

Cell material from different phases in a growth curve was analyzed for major elements and chlorophyll a content. The ranges in percent of the dry weight were 50-80%, 4.4-8.8%, 0.24-3.0%, and 0.20-3.23% for C, N, P and chlorophyll a respectively. The dry weight varied correspondingly 2.3 times. A N/P-quotient of about 10 (by weight) was found to be a critical level for Selenastrum when these elements were the most limiting nutrients for growth. 31 different organic N-compounds were tested as single N sources in growth experiments with Selenastrum. At least 19 of them could be used - to varying extents - for algal growth. (Abstract-Author).

0017

Claesson, A., and A. Forsberg. 1978. Algal assay procedure with one or five species: Minitest. Mitt. Int. Ver. Limnol. 21:21-30.

algae, Selenastrum capricornutum, Ankistrodesmus falcatus, Chlorella homosphaera, Euglena gracilis, Scenedesmus quadricauda, AGP, limiting nutrients, lake waters, toxicity, wastewater, bottle test, Minitest, algal assay.

Two algal assay methods (batch), one with Selenastrum capricornutum and one with this species together with Ankistrodesmus falcatus, Chlorella homosphaera, Euglena gracilis, and Scenedesmus quadricauda mixed on equal cell volume basis, are described and compared. Selenastrum capricornutum is routinely used for determining the maximum algal growth potential (AGP) and the limiting nutrients by enrichment experiments in lake water. The mixed algal assay method is used to estimate AGP-values and to indicate toxic effects in wastewater.

Different ways to prepare the algae before the assays are described and discussed.

The tests are performed in small plastic tubes containing a culture volume of 2.5 ml.

Different conditions influencing the growth of the algae were studied.

Examples of results from some treatment plants and some different lakes using the two algal assay procedures are given, together with a comparison of the two methods. (Summary-Author).

0017-a

Claesson, A., and S.-O. Ryding. 1977. Nitrogen-a growth limiting nutrient in eutrophic lakes. Prog. Water Tech. 8(4/5):291-299.

algal assay, nitrogen, chlorophyll a, lake waters, N:P ratio, limiting nutrients, AGP, algae, Selenastrum capricornutum.

Water chemistry and algal assays in surface water (0-2 m) are presented from five eutrophic lakes, all recipients for sewage effluent. The lakes were studied intensively during May-October 1973.

Some common features in water chemical aspects were: a decline in inorganic-N during summer, PO_4-P always in excess, and a high chlorophyll a content throughout the period. The more productive lakes, however, exhibited a high N supply even during intensive algal growth. The total-N:total-P ratio was very low for most of the test period giving N limited algal growth with the test alga Selenastrum capricornutum. A N:P ratio of 15:1 in the lake water seemed to be a critical value concerning N- or P-limitation for this test alga. The sum of lake- and algal assay chlorophyll was used to indicate the algal growth potential (AGP) in lakes, and is proposed as a simple type of "trophic index".

The lakes presented have been chosen to illustrate the variability amongst lakes where N is the most limiting nutrient. In the less productive ones N probably influenced the production level to a larger extent than in the more eutrophic ones, where other factors such as light, C-deficiency, or zooplankton predation mainly limited phytoplankton growth. (Summary-Author).

0018

Clesceri, N. L., G. C. McDonald, I. S. Kumar, and W. J. Green. 1973. Organic
    nutrient factors affecting algal growths. Ecol. Res. Series. EPA-660/3-
    73-003. 302 p.

wastewater, lake waters, organics, bottle test, algae, Microcystis aeruginosa, Anabaena flos-aquae, Selenastrum capricornutum, algal assay.

Effects of wastewater organic fractions on the growth rate of Selenastrum capricornutum and Anabaena flos-aquae were investigated. Effluent from a conventional activated sludge facility was membrane filtered, freeze-dried, and gel fractionated. Apparent molecular weights (AMW) were assigned to the appropriate fractions. These and organic carbon data showed 69% of the effluent organics had an AMW less than 700.

Absorbancies and regression analyses within algal exponential growth phases demonstrated the control growth rate for Selenastrum was 0.43 and for Anabaena was 0.34. Selenastrum growth rates were monitored using Lake George water as the diluent for the media employed. An inhibition in growth occurred. Halving the nitrogen concentration in modified Gorham's had no significant effect on growth rate.

In concentrating organics from natural water (Lake George and Saratoga Lake), raw sewage, and sewage effluent, thin film evaporation was preferred when using natural waters whereas freeze-drying was advantageous when working with sewage samples. Also, the soluble organic component in municipal wastewater was characterized and the effect of chemical-physical treatment on it has been shown. (Abstract-Author).

0019

Condit, R. J. 1972. Phosphorus and algal growth in the Spokane River.
    Northwest Sci. 46(3):177-189.

phosphorus, algae, Selenastrum capricornutum, phytoplankton, limiting nutrients, rivers, bottle test, nitrogen, manganese, algal assay.

The green algal form, Selenastrum capricornutum, proved to be an adequate and responsive test organism for natural water bioassays when properly acclimated. The growth parameters obtained from control flasks indicate that during the test periods, the total productivity and algal growth rates in the natural waters increase as the river progresses through the city. During the spring, high runoff provided adequate nutrients to promote algal growth in bloom proportions. The large biomass reduced P concentrations to limiting levels in the river. The especially high algal production in the lower river was due in part to the added P made by the wastewater treatment plant above the Bowl and Pitcher Bridge. Dissolved orthophosphate levels at this station were sufficiently high to cause inhibition to algal growth rates when additional P was introduced in the bioassay. The August bioassay indicated that productivity was governed by factors other than limiting concentrations of P. Nitrogen was found to be limiting in Porcupine Bay while Mn appeared to be a limiting factor in Sullivan Road waters. (Abstract-Biological Abstracts, Inc.).

0020

Cowen, W. F., and G. F. Lee. 1976. Phosphorus availability in particulate materials transported by urban runoff. J. Water Pollut. Control Fed. 48(3)580-591.

nutrients, phosphorus, urban runoff, algal assay, Selenastrum capricornutum, algae.

Samples of urban runoff were analyzed for dissolved reactive phosphorus, total soluble phosphorus, particulate phosphorus, and total phosphorus. Particles were isolated by membrane filtration and extracted with acid, base, and anion exchange resins, and the inorganic phosphorus in the extracts were compared with the particulate phosphorus extracted. Ranges of group mean values for various urban land uses were 33 to 46, 22 to 27, and 13 to 17 percent of particulate phosphorus for acid, base, and resin extractions, respectively. Bioassays with Selenastrum capricornutum showed a range of 8 to 55 percent of particulate phosphorus available to the algae. Long-term aerobic incubations of unfiltered runoff with resin generally showed the same percentage of particulate phosphorus extractable by resin as did short-term extraction. At most 30 percent of the particulate phosphorus should be considered available to algae in the receiving water. (Abstract-JWPCF).

0020-a

Cowen, W. F., and G. F. Lee. 1976. Algal nutrient availability and limitation in Lake Ontario during IFYGL, Part I. Available phosphorus in urban runoff and Lake Ontario tributary water. U. S. Environmental Protection Agency, Duluth, Minn., Ecological Research Series, EPA-600/3-76-094a. 218 p.

urban runoff, rivers, nutrients, phosphorus, algal assay, bottle test, algae, *Selenastrum capricornutum*.

Urban runoff, precipitation, and river samples from Madison, Wisconsin and New York state were analyzed for various phosphorus forms and the portion of each phosphorus form available for algal use. Total phosphorus, soluble phosphorus, particulate phosphorus, and soluble reactive forms were measured. In addition, acid extractable, base extractable, and anion exchange resin extractable inorganic phosphorus was determined on the particulate fractions.

Algal assay procedures were used to assess portions of the various phosphorus fractions available for *Selenastrum capricornutum* growth. Availability of particulate phosphorus in urban runoff from Madison, Wisconsin was highly variable ranging from 8 to 55 percent. Genesee River basin urban runoff had from less than 1 to 24 percent of its particulate phosphorus available. Particulate phosphorus from the Niagara, Genesee, Oswego, and Black Rivers showed only 6 percent or less available to this alga. Autoclaving the samples increased the amount of particulate phosphorus available. Precipitation samples usually showed less than 9 percent of the total phosphorus available to *Selenastrum capricornutum*. Total phosphorus available for algal growth from New York tributaries was highly variable.

About 39, 24, and 15 percent of particulate phosphorus in urban runoff from Madison could be extracted by acid, bases, and anion exchange. Results from urban areas in the Genesee River basin in New York were similar. Resin extractions in long-term aerobic dark incubations produced results similar to short-term tests, indicating that physical and chemical rather than microbial mineralization processes were probably the key factors regulating the release of inorganic P from the runoff particules to the solution phase. (Abstract-Author).

0020-b

Cowen, W. F., K. Sirisinha, and G. F. Lee. 1977. Algal nutrient availability and limitation in Lake Ontario during IFYGL, Part II. Nitrogen available in Lake Ontario tributary water samples and urban runoff from Madison, Wisconsin. U. S. Environmental Protection Agency, Duluth, Minn., Ecological Research Series. EPA-600/3-77-045. 88 p.

rivers, nutrients, nitrogen, algal assay, bottle test, algae, *Selenastrum capricornutum*, *Anabaena flos-aquae*, *Microcystis aeruginosa*.

Samples of water from the Niagara, Genesee, Oswego, and Black Rivers were collected from March to June, 1973. The samples were analyzed for nitrogen forms and were incubated in darkness under aerobic conditions to promote mineralization of soluble inorganic nitrogen from the organic nitrogen in the samples. The amounts of ammonia and nitrate were determined as a function of the time of incubation. Generally, over 50 percent of total nitrogen present in these river samples was immediately available for algal growth or potentially available after mineralization by bacteria. The results were highly variable from each tributary and no single value could be selected from the data obtained to describe the availability of total nitrogen in a given river. (Abstract-Author).

0020-c

Cowen, W. F., K. Sirisinha, and G. F. Lee. 1978. Nitrogen and phosphorus in Lake Ontario tributary waters. Water, Air, and Soil Pollut. 10(3):343-350.

rivers, streams, algal assay, phosphorus, nitrogen, nutrients, lake waters, algae, *Selenastrum capricornutum*.

During the spring of 1972 through the spring of 1973 samples of rivers tributary to Lake Ontario and streams in the Genesee River basin (New York) were analyzed for N and P forms by chemical methods, then incubated in darkness or bioassayed with algae to estimate the percentage of total N, organic N, total P or particulate P which could eventually become available for algal growth in Lake Ontario. The total available P in the river water samples could be estimated by adding to the soluble orthophosphate 0.2 of the difference between the soluble orthophosphate and the total phosphate. The total available N can be estimated from the sum of the inorganic N (NH_3 and NO_3^-)

plus 0.5 times the total organic N concentration of the river water sample. (Abstract-Author).

0021

Czygan, F. C. 1970. Studies on the importance of the biosynthesis of secondary carotenoids as a taxonomic character in green algae. Arch. Microbiol. 74(1):77-81.

biosynthesis, carotenoids, algae, Selenastrum gracile (strain 278-2), Chlorella fusca (strain 211-8b).

Under certain extreme conditions, i.e. desiccation and UV-irradiation, cells of the green chlorococcalean alga Selenastrum gracile (strain 278-2), which normally produce secondary carotenoids (esters of astaxanthin and canthaxanthin) under nitrogen-deficient conditions, will be changed to forms which are not able to biosynthesize such polyenes. The loss of this taxonomic character will recover after 10-12 transfers in fresh culture medium. In contrast, forms of Chlorella fusca (strain 211-8b) obtained as above do not revert to the wild type producing secondary carotenoids, not even after 20 transfers. (Summary-Author).

0022

Denison, J. R. 1974. Limiting nutrient tests used in an investigation of factors controlling phytoplankton development. Water Treat. Exam. 23(1): 52-75.

limiting nutrients, reservoirs, algal assay, ecological factors, phytoplankton, algae, Selenastrum capricornutum.

Data are presented showing seasonal variations of phytoplankton in four Manchester reservoirs and relationships are sought with some ecological factors controlling their growth. Selenastrum capricornutum was used as a test species in bioassays to determine the availability of growth-limiting algal nutrients and to quantify the biological response to changes in concentrations of these nutrients. Further study is required before precise interpretation of the bioassay results can be provided, in relation to the naturally occurring algal populations, but the technique promises to be a valuable and versatile tool for studying specific algal problems encountered in waterworks practice. (Abstract-Author).

0022-a

Dye, C., J. Hand, D. Jones, and L. Ross. 1978. Determination of critical nutrient levels in Florida lakes using algal assays. Florida Dept. Envr. Reg., Preliminary Report.

algal assay, lake waters, nutrients, eutrophication, agricultural runoff, chlorophyll a, nitrogen, phosphorus, limiting nutrients, N:P ratios, *Selenastrum capricornutum*, bottle test, algae.

The preliminary report is of a study conducted on 43 lakes in Florida. Samples collected from the lakes were analyzed for chlorophyll *a* content, total nitrogen, total phosphorus, and other chemical parameters. A bottle test was conducted, using the alga *Selenastrum capricornutum*. The results are given as critical chlorophyll *a* versus dry weight; and, limiting nutrients (phosphorus was found to be the primary limiting nutrient). Twenty-seven of the 43 lakes were found to have factors other than nitrogen and phosphorus levels, that limited algal growth. (Abstract-Leischman-EPA).

0023

Ferris, J. J., S. Kobayashi, and N. L. Clesceri. 1974. Growth of *Selenastrum capricornutum* in natural waters augmented with detergent products in wastewaters. Water Res. 8(12):1013-1020.

phosphate, detergents, wastewater, lake waters, bottle test, algae, *Selenastrum capricornutum* Printz, algal assay.

A determination of whether the removal of phosphate builders from detergents would modify the ability of domestic secondary treated sewage effluent to stimulate the growth of a test alga (*Selenastrum capricornutum* Printz) in receiving waters alone and augmented with detergent products was made. The lakes used as sources of test waters were located in northeastern New York state and possessed total phosphorus concentrations ranging from *ca*. 0.001 to 0.04 mg P l^{-1}.

The alga experienced stimulation in all three test lake waters from secondary sewage containing detergent with phosphate or detergent without phosphate. A concentration of 60 μg P l^{-1} was sufficient to effect significant algal growth in two of the test waters; however, concentrations ranging up to 110 μg P l^{-1} did not generate such a response in the third test water. This latter result and others suggested that neither phosphorus nor other nutrients from these wastewater additions were the factors fully accountable for the observed response(s). (Abstract-Author).

0024

Filip, D. S., and R. I. Lynn. 1972. Mercury accumulation by the alga *Selenastrum capricornutum*. Chemosphere 6:251-254.

mercury, algae, *Selenastrum capricornutum*, toxicity, algal assay.

Laboratory cultures of *Selenastrum capricornutum* were used to examine the uptake of inorganic mercury. The test organism was exposed to amounts of mercuric chloride to yield 0.1 ppm of mercury. The innoculated samples were exposed to the mercury levels for two hours and then analyzed for mercury content. The authors concluded that the mercury uptake of live cells (light-incubated cultures) and formalin killed cultures were not statistically different. The uptake of mercury was concluded to be a passive absorptive process. (Abstract-Leischman-EPA).

0025

Filip, D. S., and E. J. Middlebrooks. 1975. Evaluation of sample preparation techniques for algal bioassays. Water Res. 9(5/6):581-585.

pond water, membrane filtration, mesotrophic, eutrophication, algal assay, pretreatment, feedlot runoff, algae, *Selenastrum capricornutum*, bottle test.

The chemical and biostimulatory properties of mesotrophic pond water and eutrophic feedlot runoff were examined after subsamples of each were separately subjected to standard membrane filtration and autoclaving pretreatments for bottle test bioassays. Correspondingly separate subsamples were exposed to ultraviolet light to determine its effectiveness as a possible pretreatment. All three pretreatments fulfilled the requirement of destroying or removing indigenous algae, but each technique substantially altered the water chemistry changing the bioassay results. Filtration removed phosphorus; autoclaving raised the pH value, precipated salts and eliminated dissolved carbon dioxide; and ultra-violet light oxidized organic and inorganic nitrogen compounds. (Abstract-Author).

0026

Fitzgerald, G. P. 1970. Aerobic lake muds from the removal of phosphorus from lake waters. Limnol. Oceanogr. 15(4):550-555.

phosphate, phosphorus, sorption rates, sediments, aerobic, phytoplankton, Cladophora sp., algae, Selenastrum capricornutum, lake waters, limiting nutrients, algal assay.

Phosphorus-limited Selenastrum and Cladophora sp. will respond by growth or changes in extractable PO_4-P to as little as 0.02 mg PO_4-P in solution; these same species did not respond when exposed for a period of 1 or 2 weeks to as much as 2 mg of phosphorus as lake muds under aerobic conditions. Studies of the rate of sorption of phosphorus by lake muds, under aerobic conditions, from two lakes and from three depths in one lake, indicated that as little as 0.4 g (dry wt.) of mud could sorb about 0.05 mg PO_4-P in less than 30 min. These findings suggest that the sorption of phosphorus by lake muds under aerobic conditions can be used to remove phosphorus from lake water. (Abstract-Author).

0027

Fitzgerald, G. P. 1972. Bioassay analysis of nutrient availability, p. 147-165. In H. E. Allen and J. R. Kramer [eds.], Nutrients in natural waters, John Wiley & Sons, Inc., New York.

algal assay, nutrients, phosphorus, nitrogen, algae, Microcystis aeruginosa, Chlorella pyrenoidosa, Selenastrum capricornutum, Anabaena flos-aquae, ammonia, lake waters, Spirogyra sp., Cladophora sp.

The paper is a full-length chapter within the book, Nutrients in natural waters, and consists of an in-depth discussion of the nutrient analyses for bioassays. No abstract is provided. (Abstract-Leischman-EPA).

0027-a

Fitzgerald, G. P. 1974. Applications of growth and sorption algal assays. U.S. Environmental Protection Agency, Washington, D. C., Ecological Research Series EPA-660/3-73-023. 175 p.

lake waters, nutrients, sorption, algal assay, phosphorus, agricultural runoff, wastewater, water quality, algae, Selenastrum, Microcystis, Anabaena, duckweed, Lemna minor, phytoplankton.

The availability of nutrients in selected Wisconsin lakes was measured in laboratory studies utilizing both sorption and growth algal assays. These tests were conducted to evaluate contributions of phosphorus to the Madison area lakes from septic tanks, agricultural runoff, and urban drainage and to measure changes in the nutritional status of six lakes which were manipulated for water quality improvement by nutrient inactivation or hypolimnetic aeration. Characteristics of the assay techniques are discussed and results are compared to chemical determinations of plant nutrients. (Abstract-Author).

0027-b

Fitzgerald, G. P. 1975. Are chemicals used in algae control biodegradable? Water and Sewage Works 122(5):82-85.

pollution, biodegradation, toxicity, algistatic, algicidal, algal assay, copper, mercury, ammonia, silver, algae, Selenastrum capricornutum, Microcystis aeruginosa, Chlorella pyrenoidosa, Anabaena, Gloeotrichia, Oscillatoria.

Data collected during tests performed over the last 11 years have indicated that the algicides that have been used to control the growth of problem-causing algae, products containing copper, silver, mercuric chloride, phenyl mercuric acetate, and quaternary ammonium compounds, do not pose a threat to aquatic environments if used at the minimal concentrations required to be effective, because these concentrations are biodegraded by the treated algae. However, all chemicals to be added to aquatic environments should be evaluated, since at least one compound --methyl mercuric chloride--is not detoxified by the action of treated algae and its use as an algicide would pose a threat to the general environment. (Abstract-Author).

0027-c

Fitzgerald, G. P. 1975. Factors affecting the Algal Assay Procedure. Report for Office of Research and Monitoring, U. S. Environmental Protection Agency, Washington, D. C. Project No. P5J11912-J. 31 p.

algal assay, nutrients, bottle test, nitrogen, phosphorus, carbon, light intensity, limiting nutrients, growth rates, standing crop, maximum yield, fluorescence, lake waters, algae, Selenastrum capricornutum, phytoplankton, Cladophora, diatoms.

Evaluations of the Algal Assay Procedure (AAP) have demonstrated its value for determining the level of nutrients in water samples which is available for the growth of algae as contrasted to chemical analyses of total nutrient contents. The maximum specific growth rate, μ max, has been shown not to be affected by the supply of N, P, or carbon when tests are carried out using the suggested AAP. The maximum yield of algae is affected by the initial concentration of N and P (and Fe, Ca, Mg, K, and S in other nutrition studies). The maximum yield is not affected by the supply of carbon in normal AAP cultures (foam or cotton plugs), but increased incubation times might be required for the maximum yield to be attained if comparatively concentrated nutrient solutions are used. The inoculum levels of algae suggested in the AAP are sufficiently low to make use of fluorometry as a measurement of the growth of algae in relatively dilute culture media. If cell counts or absorbance measurements are to be used to follow the growth of cultures, higher initial cell densities may be employed. The μ max of algae cultures is influenced by the light intensity, but maximum yields merely require longer incubation periods if less than suggested light intensities are used. The applications of the AAP with the suggested algal species or *in vivo* algae have demonstrated its value in determining which algal nutrient will limit the growth of algae in water samples. (Abstract-Author).

0027-d

Fitzgerald, G. P., S. L. Faust, and C. R. Nadler. 1973. Correlations to evaluate the effects of wastewater phosphorus on receiving waters. Water and Sewage Works. 120(1):48-55.

phytoplankton, *Rhizoclonium* sp., *Cladophora* sp., *Spirogyra* sp., algae, *Selenastrum capricornutum*, phosphorus, rivers, wastewater, algal assay, bottle test, nitrogen, iron, lake waters, sorption, extraction, chemical analysis.

Studies were conducted to determine the amounts of phosphorus that were available for algal growth in lakes and rivers within Wisconsin. An algal assay and a chemical analysis were the methods used to measure phosphorus levels. Within the algal assay procedure, two tests were conducted, the one-day sorption-extraction test and the long-term (12 days more) bottle test. The results from the two tests were in agreement, as relating algal growth to phosphorus availability. The authors did conclude that the long-term assay be used in conjunction with the short-term and the chemical analysis, as a check system of the phosphorus availability for growth. The methods of measuring available phosphorus were comparable; the authors concluded that either method could be used to measure phosphorus concentrations available for algal growth. The authors also concluded that data from the tests and analyses could be used to determine whether water systems contained excess phosphorus. (Abstract-Leischman-EPA).

0027-e

Fitzgerald, G. P., M. S. Torrey, and G. C. Gerloff. 1975. Green Bay self-purifies algal nutrients. Water and Sewage Works. 122(6):58-61.

pollution, wastewater, nutrients, phosphorus, nitrogen, water quality, algal assay, bottle test, algae, Selenastrum capricornutum, phytoplankton, Cladophora, rivers, lake waters.

Algal assays were used to determine the effects of nutrient loadings in the Fox River-Green Bay system. The studies were designed to aid in evaluating and determining the effects of pollutants already present or being added to the water system. Field assays were conducted with the green alga Cladophora; Selenastrum capricornutum, a green alga, was used in bottle tests to determine effects of removal of phosphorus from the waters. The authors concluded that if Green Bay were to be improved in terms of nutrient removal (self-purification), the results would be effective in reducing algal growth in the Great Lakes area. (Abstract-Leischman-EPA).

0027-f

Forsberg, A. 1974. Algal assay with drainage water from waste disposal. Vatten 30(1):79-83. In Swedish.

pollution, wastewater, phosphorus, inhibition, algae, Ankistrodesmus falcatus, Chlorella homosphaera, Scenedesmus quadricauda, Selenastrum capricornutum, Euglena gracilis, algal assay.

During spring 1972 some unknown pollution in the water of the River Motala Strom gave the drinking water of Norrkoping a bad taste. Drainage water from a big waste dump was discussed as a possible source for this incident.

In order to study the effect of drainage water on algal growth, samples were taken during a one year period, one week (5 days) every month, from a brooklet receiving this drainage water. Mixed week samples were also analysed for PO_4-P, total-P, COD and pH.

The algae were exposed to 0, 2.5, 10, 25 and 40% of the test water. The incubation period was 7 days. The results are expressed as relative growth in relation to the standard, an artificial nutrient solution without addition of test water. Samples from the brooklet showed algal growth in proportion to the amount of test water added. No toxic effects could be detected. The corresponding tests with water from the drainage pond gave inhibitory effects at 25% and 40%. (Modified translated Summary-Author).

0028

Forsberg, A. 1978. Research on recovery of polluted lakes: Chemical and biological methods for monitoring wastewater effluent quality. Abstr. Uppsala Diss., ACTA Univ. Upsaliensis No. 458, Uppsala, Sweden. 28 p.

wastewater, toxicity, phosphate, phosphorus, ammonia, nitrite, nitrate, algae, Ankistrodesmus, Chlorella, Scenedesmus, Selenastrum, Euglena, Minitest, algal assay.

The quality of effluent water from chemical treatment plants has been shown to vary considerably. Thus it is essential to conduct frequent sampling to obtain a reliable basis for estimating nutrient loadings. The Minitest, a handy method for sampling and analyzing phosphate, phosphorus, total phosphorus, chemical oxygen demand, ammonia, nitrite, nitrate, organic nitrogen and other constituents if required, is presented. This procedure also involves the use of a mixture of five algal species, four green algae and one flagellate, for biological control of the effluent quality. Toxic effects of wastewater effluent have been shown with a 40 times dilution (2.5% wastewater). Besides description of fertilizing capacity and presence of toxic compounds the algal assay also provides an opportunity to estimate the availability of nutrients, which for instance is of interest in comparisons between different treatment processes. (Abstract-Author).

0028-a

Forsberg, A. 1978. Algal growth response and variation in cell composition in cultures of a mixed algal system under varying nutrient conditions.

No. V, Publ. in Abstr. Uppsala Diss., ACTA Univ. Upsaliensis No. 458, Uppsala, Sweden. 14 p.

algal assay, nitrogen, carbon, phosphorus, chlorophyll *a*, algae, *Ankistrodesmus falcatus* (Corda) Ralfs., *Chlorella* sp., *Scenedesmus quadricauda* Turpin em. Chod., *Selenastrum capricornutum* Printz, *Euglena gracilis* Klebs., nutrients.

A mixed algal system consisting of five algal species (*Ankistrodesmus falcatus* (Corda) Ralfs, *Chlorella* sp., *Scenedesmus quadricauda* Turpin em. Chod., *Selenastrum capricornutum* Printz and *Euglena gracilis* Klebs) could utilize at least 20 out of 31 organic nitrogen compounds as the only nitrogen source. Analyses of cell contents of carbon, nitrogen, phosphorus, and chlorophyll *a* gave the ranges 38-64%, 2.25-4.05%, 0.25-1.6% and 0.19-1.7% of dry weight respectively. From the lower figures of nitrogen and phosphorus in these ranges, cell yield coefficients could be estimated to be $Y_N = 44$ and $Y_P = 400$. The corresponding values obtained with gradient experiments gave about twice the yields, $Y_N = 90$ and $Y_P = 775$. (Abstract-Author).

0029

Forsberg, C. G. 1972. Algal assay procedure. J. Water Pollut. Control Fed. 44(8):1623-1628.

wastewater, nutrients, AGP, algae, *Selenastrum capricornutum*, *Euglena gracilis*, *Ankistrodesmus falcatus*, *Scenedesmus quadricauda*, bottle test, algal assay.

A new algal assay procedure using small samples, small culture volumes, and short incubation periods has been developed. The algal growth potential of wastewater effluents has been tested in a standard recipient solution including test algae. The method permits tests with several algal species in the same standard. The difference between the maximum algal growth in the standard recipient solution with and without the addition of wastewater effluent was taken as a measure of the algal growth potential of the effluent. Preliminary results obtained with effluents sampled at some Swedish wastewater treatment plants are presented. The growth of *Selenastrum capricornutum* can be about 90 percent lower in chemically treated wastewater than in effluent from biological treatment. (Abstract-JWPCF).

0029-a

Forsberg, C., and A. Claesson. 1974. Recovery of polluted lakes. Algal growth limiting nutrients and algal assay (Selenastrum) chlorophyll. Vatten 30(1):84-95. In Swedish.

wastewater, water quality, AGP, chlorophyll, N:P ratios, phosphorus, nitrogen, chelation, trace elements, algal assay, algae, Selenastrum, limiting nutrients.

This paper deals with algal assays performed parallel to the chemical analyses with water collected from August--October, 1972. All algal assays were performed with glass fiber filtered (Whatman GF/c, 0.45 μ) surface water, 0--2 m. The algal assay procedure is essentially the same as the bottle test method, though several modifications have been performed. Some are in accordance with ideas previously presented e.g. small culture volumes. Also the procedure for the enrichment experiments has been simplified. The tubes were prepared beforehand with solutions of phosphorus, nitrogen, etc. in small volumes. After evaporation the salt was resolved in the test water. A short preliminary report of "Mini-test" with lake water has been presented.

The growth of the test alga, Selenastrum capricornutum, was determined as the volume of the cells. The algal volumes have also been expressed as chlorophyll. The correlation between the volume of Selenastrum and the chlorophyll content (obtained by the acetone method) of the cells gave the correlation coefficient r= +0.99 (n=25).

Enrichment experiments were performed in order to study algal growth limiting nutrients. Results from Lake Kyrkviken show the variation of the growth potential of surface water from week to week. Each sample tested was a mixed week sample based on three samples/week. Enrichment with nitrogen had a marked effect. Monthly average values of the growth potential of water from Lake Sillen are given along with the results of tests enriched with phosphorus, nitrogen, chelating agent and trace elements.

The proportions between the lake water chlorophyll and the algal assay (Selenastrum) chlorophyll are illustrated. The primary growth limiting nutrients are also indicated. The sum of the natural lake water chlorophyll and the algal assay chlorophyll has been related to the ratio total-N:total-P. Lake Frösjön and Lake Glaningen showed special features. The former are polluted by a detergent factory which can explain the diverging conditions in this lake water. The low chlorophyll content of Lake Glaningen can depend on special conditions giving rise to big masses of very small easily consumed algae. In any case the algal assay indicated no toxic effects in this water.

The sum of the two chlorophyll fractions mentioned above was correlated to the content of total-P. Below phosphorus values of 0.05 mg/l phosphorus was the growth limiting nutrient. Above 0.1 mg/l nitrogen played the corresponding role. Between these values phosphorus, nitrogen or chelating agent could be the primary growth limiting factor. The lakes belonging to the last mentioned group, with total-P values between 0.05--0.1 mg/l will be expected to give the most rapid answer on advanced waste water treatment for phosphorus removal. (Modified translated Summary-Author).

0030

Forsberg, C. G., and A. Forsberg. 1972, Algal growth potential test improves sewage effluent control. Ambio 1(1):26-29.

wastewater, algal assay, phosphorus, algae, phytoplankton, Selenastrum capricornutum, Euglena gracilis, Ankistrodesmus falcatus, Oocystis submarina var, variabilis, Scenedesmus quadricauda, AGP, toxicity.

A new mixed algal assay procedure for monitoring sewage effluent is described. Results from routine tests of effluents at treatment plants are given. Five algae were used. Effluent from one plant showed daily rhythm in algal production, low values during the summer vacation, and disturbed treatment efficiency due to incoming oil. The correlation coefficient for total-P and algal volume was + 0.81; for PO_4-P and algal volume it was + 0.99. Toxic effects were noted. (Abstract-Ambio).

0030-a

Forsberg, C., and E. Hokervall. 1971. An algal assay procedure of value for control of sewage effluents from treatment plants in Stockholm. Vatten 27(1):51-57. In Swedish.

wastewater, algal assay, AGP, algae, Selenastrum capricornutum, chemical flocculation.

This paper presents the first results with a new algal assay procedure of value for control of sewage effluents from treatment plants with chemical flocculation. In July, 1970, about 30 chemical treatment plants were functioning in Sweden and more than 200 more were projected. Since the aim of chemical flocculation is to reduce the fertilizing power of the effluents, the idea of using an algal-assay procedure has been tested at the algal test laboratory of the Institute of Physiological Botany, Uppsala. A brief description of the method is presented together with some results obtained with effluents from three treatment plants in Stockholm.

Small samples of the effluents were frozen quickly and then sent by regular mail while still frozen, in special boxes. After thawing and filtering, the samples were diluted in a recipient solution with ionic concentrations of the same order as ordinary lakes. The recipient solution also includes the test alga, Selenastrum capricornutum (a mixed algal standard will be developed later). To this standard the sewage effluent was added at concentrations of 5 o/o, 2.5 o/o or lower. The difference between the maximal algal growth in the standard recipient with and without addition of sewage effluents were calculated. The volume of algae produced was measured by electronic particle counting. The new method requires small volumes of sample, about 5 ml, small culture volumes, 1 ml, and short test periods, 3--4 days.

Algal-growth potential (AGP) values from some tests with sewage effluents from the chemical-biological treatment plant at Eolshäll are presented. The AGP values were transformed from the algal volume figures. AGP-values between 0.5--1.5 were obtained when no disturbance of the treatment was detected. During the last part of April the spring flood was the reason for the poor treatment which gave increased AGP-values. At the end of September and beginning of October the values reached a level of about 8 which corresponds to biological treatment only. This depended on incoming oil (September 28--29) and technical errors (first half of October) with the equipment for the chemical flocculation. The biological treatment plants of Loudden and Akeshov-Nockeby were tested during the end of their operation as biological treatment plants only. The plant at Akeshov-Nockeby gave values of the same order as other biological treatment plants tested, about 8, while the plant at Loudden reached higher values. On December 1, 1970 the plant at Akeshov-Nockeby started with chemical-biological treatment and for the last period tested, December 3--9, all AGP values were between 0.5--2. (Modified translated Summary-Author).

0030-b

Forsberg, C., and E. Hokervall. 1972. Algal growth potential test (AGP-test) of sewage effluent. Vatten. 28(1):17-26. In Swedish.

wastewater, AGP, algal assay, nutrients, nitrogen, phosphorus, phosphate, algae, Ankistrodesmus falcatus, Euglena gracilis, Oocystis submarina var. variabilis, Scenedesmus quadricauda, Selenastrum capricornutum.

From February 1971 the efficiency of sewage treatment at the chemical and biological treatment plant at Akeshov-Nockeby (230,000 pe.), Stockholm, has been continuously tested using a new algal assay procedure. Results from 240 day-samples together with chemical data are presented.

The nutrient content of unfiltered sewage effluent was tested after dilution to 2.5 o/o with a laboratory recipient solution. This solution includes the test algae, five different species: Ankistodesmus falcatus, Euglena gracilis, Oocystis submarina var. variabilis, Scenedesmus quadricauda and Selenastrum capricornutum. These species were chosen for their excellent growth in dilute sewage. The difference between maximal algal growth in the standard with and without the addition of sewage effluent was taken as a measure of the algal growth potential (AGP). The method for the algal assay procedure, which will subsequently be described in more detail, requires comparatively short test periods, small volumes of sample (5 ml), small culture volumes (2.5 ml) and employs disposable plastic culture tubes.

Chemical analyses of the day-samples representing Tuesday to Wednesday were performed, parallel to the algal tests at The Stockholm City Municipal Services Department, Construction Division. Phosphate-phosphorus was analysed at the algal test laboratory as day-average per week for 7 mixed day-samples.

The algal volumes obtained during 8 months are given. During these months the treatment plant showed a rhythm. The high values in June are due to incoming oil, which disturbed the last (biological) step in the treatment. During July and August some samples gave no algal production at all indicating some inhibitory effect of the effluent. The phosphate content was extremely low during the vacation period, with some average values per week below 0.10 mg/l. In nonindustrial effluent from other treatment plants tested, the same results were obtained when the Al content was comparatively high, 1--2 mg/l, with the highest value of 4 mg/l. As preliminary studies have indicated that algal growth can be retarded by comparatively low amounts of Al (unpublished), one reason for the low production in the effluent, as observed during July and August, could be that the dosage of Al-sulphate in the treatment plant was too high in relation to the existing, low phosphate content. Correlations for monthly average values for samples representing Tuesday--Wednesday were observed between total-phosphorus, phosphate-phosphorus and algal volume produced. The correlation coefficients were +0.81 and +0.88, respectively. Positive correlations also were obtained between the monthly average values of BOD_5, suspended material and total-phosphorus with the correlation coefficients of +0.78 and +0.89 respectively. The relations between total-nitrogen, total-phosphorus and algal volume produced are illustrated. The correlation between algal volume, total-phosphorus and BOD_5, is also outlined.

The average algal volume produced during the whole period was 425 mm^3/l (n=240). The corresponding phosphate value was 0.25 mg/l. The biggest outflow of phosphate-phosphorus occurred during week 24, when 500 kg was transported by 760,000 m^3 effluent. During week 31 only 60 kg was transported by 700,000 m^3.

As a result of this investigation further effort will be directed into decreasing the fertilizing capacity of the effluent and to eliminating the high values obtained in the middle of the week. During the vacation period in 1972 the dosage of Al-sulphate will be lowered from that of the normal months. (modified translated Summary-Author).

0031

Forsberg, C. G., S.-O. Ryding, and A. Claesson. 1975. Recovery of polluted lakes: A Swedish research program on the effects of advanced wastewater treatment and sewage diversion. Water Res. 9(1):51-59.

Minitest, wastewater, algae, Selenastrum capricornutum, chlorophyll a, phosphorus, nitrogen, lake waters, algal assay.

In order to elucidate the effects of the effluents from advanced wastewater treatment plants for phosphorus removal, the National Swedish Environment Protection Board during 1972 started a program for analyzing the conditions in a number of different recipient lakes. This study of recovery of polluted lakes also includes some recipient lakes from which sewage effluent will be completely diverted. This paper presents the program, some new methods used and the treatment plants and recipient lakes involved. The project, including 18 lakes and 15 sewage treatment plants is running in cooperation with 13 Provincial Governments and 16 Municipalities. The efficiency of the sewage treatment plants is continuously monitored by a new method called Minitest, and the nutrient loadings from these plants as well as from other sources are calculated.

As the water quality of many recipient lakes showed great variations during the vegetation period, a need for a frequent sampling exists in these lakes.

In order to simplify the sampling procedure sampling on the shore (after pumping water from the sampling point) has been introduced. Data obtained after analyzing samples taken from a boat and samples from the same area taken on the shore after pumping through a 600 m plastic tube showed good agreement.

The lake water samples are preserved by freezing. Analyses after rapid thawing showed that this method can be used also when determining from cell material easily leaking ions such as PO_4, NO_3, etc.

The sewage treatment plants, the recipient lakes and some background data are presented. The water quality of the lakes is illustrated with the correlation between chlorophyll a and suspended solids. The correlation coefficient = + 0.88. The first sign of lake recovery has been observed after an 85 percent reduction of the waste phosphorus load. Post-precipitation (Al-sulphate) decreased the yearly load from 2000 kg P to 300 kg, which gave a 50 percent reduction of the P content of the recipient water. Also, the chlorophyll values decreased.

The role of nitrogen and phosphorus as algal growth limiting nutrients has been studied by using an algal assay procedure. N limited the growth of *Selenastrum capricornutum* in 2/3 of the samples. In filtered lake water P was the growth limiting nutrient at total-P values below 0.05 mg l^{-1}. Above 0.1 mg P l^{-1}, N played the principal role. Between these values the growth was primarily limited by P or N or chelating agents. (Abstract-Author).

0031-a

Forsberg, C., S.-O. Ryding, A. Forsberg, and A. Claesson. 1978. Research on recovery of polluted lakes. I. Improved water quality in Lake Boren and Lake Ekoln after nutrient reduction. Verh. Int. Ver. Limnol. 20(2):825-832.

wastewater, phosphorus, nitrogen, chlorophyll a, COD, algal assay, algae, Selenastrum capricornutum, water quality, nutrients.

After advanced wastewater treatment for P removal improved water quality has been observed in Lake Boren and Lake Ekoln. The P-load from the treatment plants at Motala and Uppsala has been reduced from 30 to 3 and from 100 to 20 tons yr^{-1}, respectively. In L. Boren and L. Ekoln the average chlorophyll a values for surface water have decreased by 50 o/o, from about 10 to 5 and from 20 to 10 mg m^{-3}, respectively. For lowering chlorophyll a concentration by 1 mg m^{-3} in the lakewater as an average for the summer period, the annual P-load had to be decreased by 6 and 5 tons, respectively. The P residence time was of the same order in these lakes. In both lakes the transparency as an average for the summer period has increased from 1.5 to 2 m. Further improvements can be expected. (Summary-Author).

0032

Forsberg, C., S.-O. Ryding, A. Claesson, and A. Forsberg. 1978. Water chemical analyses and/or algal assays? Sewage effluent and polluted lake water studies. Mitt. Int. Ver. Limnol. 21:352-363.

wastewater, algal assay, lake waters, chemical analysis, limiting nutrients, algae, Selenastrum capricornutum, Ankistrodemus falcatus, Scenedesmus quadricauda, Chlorella sp., Euglena gracilis, AGP, pollution.

In a Swedish research program dealing with recovery processes of polluted lakes, algal assay procedures have been routinely used for three years, for determining algal growth potential (AGP) and growth-limiting nutrients. Both sewage effluent and lake water were studied. Algal assay data and results from chemical water analyses are presented in order to illustrate and discuss the usefulness of the practical application of algal assay procedures.

After chemical treatment for phosphorus removal, the algal growth of five species (mixed culture of Ankistrodesmus falcatus, Chlorella homosphaera, Euglena gracilis, Scenedesmus quadricauda, and Selenastrum capricornutum) in sewage effluent, often showed a high correlation to the phosphate content of the test water. But, the results also indicated both growth-inhibiting and toxic compounds in the effluent. Monthly average values for nitrogen, phosphorus, chlorophyll a (chl a) and AGP expressed a chl a, are presented from three lakes. The AGP values of filtered water, expressed as chl a, were determined for 17 polluted lakes by using S. capricornutum as the test alga. In four lakes, AGP was $\leq$ 10 mg chl a/m^3. The maximum value obtained was 488 mg/m^3.

The Selenastrum test indicated N to be the most limiting nutrient in seven of 17 lakes, P in seven lakes, and N, P in the remaining three. Calculated from chemical data, N was found to be the most limiting nutrient in five lakes and P in eight lakes. In four lakes, the available P and N was calculated to give rise to the same AGP values, which means that in these lakes neither P nor N could be looked upon as the most limiting nutrient. Ratios for N:P in the lake water in relation to the role of these elements as most limiting nutrient are suggested. Values obtained for the maximum specific growth rate of Selenastrum showed tendencies to increasing growth rate with increasing degree of pollution.

Algal assays were found to be of great value for explaining situations which cannot be indicated by chemical analysis. On the other hand, many results presented today by algal assays can equally well be calculated by water chemical data. (Modified Summary-Author).

0033

Francisco, D. E., and C. M. Weiss. 1973. Algal response to detergent phosphate levels. J. Water Pollut. Control Fed. 45(3):480-489.

detergents, phosphate, wastewater, algal assay, phosphorus, eutrophication, lake waters, algae, Selenastrum capricornutum, limiting nutrients.

Algal assays were performed on treated wastewaters, some containing phosphate detergents and others containing a non-phosphate detergent. In general, phosphate detergent wastewater allowed the same growth as did non-phosphate detergent wastewater at each level of treatment. Tertiary treatment for phosphorus removal resulted in much less growth than secondary treatment. Tertiary-treated phosphorus detergent wastewater allowed significantly greater growth than the non-phosphorus wastewater at a concentration of 2 percent. Simply removing detergent phosphates from wastewater apparently will not control eutrophication, except where the receiving water is very strongly phosphorus-limited and wastewater is the principal phosphorus source, but may allow significantly better tertiary phosphorus removals. (Abstract-JWPCF).

0034

Funk, W. H., F. W. Rabe, R. Filby, J. I. Parker, J. E. Winner, L. Bartlett, N. L. Savage, P. F. X. Dunigan, Jr., N. Thompson, R. Condit, P. J. Bennett, and K. Shaw. 1973. Biological impact of combined metallic and organic pollution in the Coeur d'Alene - Spokane River drainage system. Washington State Univ. University of Idaho. OWRR (B-044 WASH and B-015 IDA). 187 p.

rivers, sediments, toxicity, zinc, algae, Selenastrum capricornutum, phytoplankton, algal assay, water quality, cadmium, lead, mercury, iron, manganese.

An investigation was made into the biological effects of metallic and organic pollution in the Coeur d'Alene - Spokane River Lake drainage system.

Water quality data show heavy inflows of nutrients during late spring into Coeur d'Alene Lake from the St. Joe and Coeur d'Alene Rivers and Plummer Creek. Although no waters devoid of dissolved oxygen have been measured, 2-4 mg/l have been measured in the southern portion of the lake. Phytoplankton productivity measurements and bottom organism identification and enumeration have shown that the lake functions at several trophic levels, meso-eutrophic to eutrophic in the southern portion, mesotrophic in the immediate area of the Coeur d'Alene River and oligotrophic in the northern portions. High metal concentrations (1000-7000 mg/kg Zn) in the bottom sediments of the middle and northern portions of the lake did not appear to substantially affect distribution of benthic chironomids or oligochaetes.

Lake sediment cores taken across the delta region of the Coeur d'Alene River penetrated through the regions of high metallic concentration and allowed an average sediment deposition rate to be calculated.

Algal toxicity tests showed the amount of Zn normally present in the Coeur d'Alene Lake and River and the Spokane River to be inhibitory to the algal test organism Selenastrum capricornutum.

The water quality of the Spokane River was shown to be of good to excellent quality in all parameters tested except for high metallic content, especially Zn. Activation and atomic absorption analysis of the metallic content in tissues of the organisms populating the Spokane River indicated that the algae were the prime concentrators of Zn, Cd, Pb, Hg, Fe, and Mn. Algae and detritus consumers such as the larvae of the caddis fly Hydropsyche and the nymphs of may fly Baetis reflected high metallic concentrations. Most higher aquatic plants showed relatively lower concentrations.

Analysis of fish tissues showed a considerably less concentration of metals than the aquatic-plants, insects, or algae. However, Zn was measured at concentrations of 80-200 mg/kg in liver tissues of several species of fishes. Fillet tissues generally contained less than one quarter of these amounts. The fish, when collected, did not appear to be under stress leading the investigators to believe that most of the metals, although present in relatively high concentration in the tissues of the organisms tested, must be in a relatively innocuous state. (Abstract-Author).

0035

Gargas, E. 1973. Preliminary statements of algal assay procedure at the Water Quality Research Institute, Denmark, p. 19-32. In Algal assays in water pollution research, Proc. Nordic Symp., 25-26 October 1972, Oslo, Norway. NORDFORSK, Secretariat of Environmental Sciences.

algal assay, bottle test, algae, Selenastrum capricornutum, Phaeodactylum tricornutum, AGP, diatoms.

A modified bottle test procedure was used to determine how algae would grow under different cultivating conditions, such as light intensity. The author found no difference in biomass (when expressed as cell number, cell volume, organic nitrogen or organic phosphorus) cultivated under different light intensities, although the cultures under the lowest light intensity showed a growth lag. The author indicated that light conditions must be considered when expressing biomass in terms of chlorophyll a content or inorganic carbon. The algal species used were Selenastrum capricornutum and Phaeodactylum tricornutum. (Abstract-Leischman-EPA).

0036

Gargas, E. 1978. The effect of sewage (mechanically, biologically, and chemically treated) on algal growth. Mitt. Int. Ver. Limnol. 21:110-124.

wastewater, algal assay, rivers, lake waters, nitrogen, phosphorus, algae, Selenastrum capricornutum.

The report is a presentation and discussion of results of algal assays conducted in a laboratory and in situ. The assays were part of an investigation on pollution of the Guden River System in 1974-75. The laboratory experiments were designed to study effects on growth rates of Selenastrum capricornutum from spikes of increasing concentrations of sewage (mechanically treated and biologically-chemically treated). Organic matter produced was measured and expressed in terms of cell numbers, dry weights, chlorophyll a content, and concentration of nitrogen and phosphorus for each concentration of sewage spike. The addition of the sewage spikes into the natural system caused an increase in phytoplankton production but the author could not conclude that the production was directly a result of increased growth rates. Another form of measurement used in the field experiments was the combination of secchi depth and chlorophyll a content. The author was able to make linear regression analyses to estimate the effect of sewage loadings on the increased chlorophyll a content. The author concludes that the algal assay could be conducted with sewage waters, as spiked and unspiked waters, to estimate the effects of sewage loading on receiving waters; however, it was not possible to estimate the effects of sewage loading on the natural phytoplankton in the field. (Abstract-Leischman-EPA).

0037

Garton, R. B. 1973. Biological effects of cooling tower blowdown, p. 284-292. In Water-1972, AIChE Symp. Ser. No. 129, Vol. 69, New York.

algae, Selenastrum capricornutum, algal assay, fish, Salmo gairdneri, morpholine, cyclohexylamine, hydrazine, zinc, phosphate, sulfates, sodium chromate, heavy metals, toxicity, nuclear power.

Toxicity was determined of a 'typical' cooling tower blowdown and of its individual components. Since blowdown composition is variable, a simulated blowdown was made using chemicals and concentrations listed in waste discharge permit applications. The mixture contained varying amounts of chromate, zinc, phosphate, sulfate, boron, sodium, ammonia, morpholine, cyclohexylamine, and hydrazine, and simulated blowdown from the Trojan nuclear plant. Separate tests were also conducted with sodium chromate, zinc, phosphate, and with blowdown without chromate. Fish (Salmo gairdneri) and an alga (Selenastrum capricornutum) were used in bioassays of the mixtures. Algal assays were conducted according to the Algal Assay Procedure Bottle Test. Fish bioassays were conducted in accordance with procedures given in Standard Methods. Algal growth was reduced by blowdown at full strength and at dilutions of 0.1. Fish were killed at dilutions of 0.1. No effect was observed at 0.032 dilution. The LC_{50} value was 0.068 dilution. With sodium chromate, algal growth was reduced at 0.139 ppm, and fish survived 96 hours at 31 ppm. Zinc phosphate had no significant effect on algal growth at 0.016 ppm. The LC_{50} for fish was 0.09 ppm zinc phosphate. Based on all the tests, zinc and chromium were identified as the toxic components in the blowdown. It is noted that synergistic effects may occur in other mixtures containing different compounds. Furthermore, the organisms used in the bioassays must be specified since toxicities may differ for different ones. (Abstract-Little-Battelle WRSIC).

0038

Gerhold, R. M. 1976. Algal nutritional bioassay of Lake Wylie, North Carolina, p. 175-220. In E. J. Middlebrooks, D. H. Falkenborg, and T. E. Maloney [eds.], Biostimulation and nutrient assessment, Ann Arbor Sci., Mich.

lake waters, algal assay, bottle test, algae, *Selenastrum capricornutum*, limiting nutrients, phosphorus, phytoplankton, *Anabaena circinalis*, *Anabaena wisconsinense*, *Microcystis aeruginosa*, *Anabaena scheremetievi*, *Anabaena sprioides*, *Raphidiopsis curvata*, *Oscillatoria tenuis*, *Oscillatoria geminata*.

The key findings from the bioassay studies, ancillary laboratory experiments, and the field observations are summarized below:

1. Phosphorus was identified as a limiting nutrient in Lake Wylie waters.

2. Overall, it appears that an approximate doubling of the mean annual available phosphorus concentration in the Lake Wylie study area would have to occur before another nutrient would become limiting.

3. The bioassays did not indicate any influence of the Allen Stream Station on the ability of the Lake Wylie study area waters to support algal growth as a function of nutrient content.

4. No measurable effects of urbanization were encountered in the study.

5. Ancillary laboratory experiments and field observations support the hypothesis that the Lake Wylie study area waters are not conducive to the overabundant growth of potential nuisance blue-green algae, and that the low hydrogen ion concentration may be prominent among the factors responsible.

6. Field observations suggest that the genus *Oscillatoria* was the most prominently represented blue-green algae during the study period. (Summary-Author).

0039

Gerhold, R. M., and R. G. Otta. 1976. Algal bioassays to evaluate a proposed cooling lake. J. Water Pollut. Control Fed. 48(10):2351-2366.

lake waters, reservoirs, algal assay, algae, Selenastrum capricornutum, nitrogen, phosphorus, nuclear power.

The primary objectives of this study of waters to be used in supplying a proposed cooling lake for a Kansas nuclear power station were to identify and quantify algal growth-limiting nutrients, compare available concentrations with chemical analyses, predict sensitivity to changes in concentrations of the limiting nutrients, measure the algal growth potentially supported by the waters, and predict the effects on algal growth of evaporative concentration of nutrients within the proposed impoundment.

The results support the following conclusions:

1. Nitrogen or phosphorus alone or in association were the limiting nutrients. Some differences in limiting nutrient relationships were noted in samples collected at different times of the year.

2. Chemical analysis of the inorganic forms such as orthophosphate phosphorus or the sum of ammonia, nitrate, and nitrite nitrogen frequently underestimated the biologically available portion of the limiting nutrient, and total phosphorus, or total nitrogen as the sum of organic and inorganic nitrogen forms generally overestimated the biologically available portion. For this reason, it was concluded that chemical analyses alone should not be substituted as estimators of biological availability.

3. Although added nitrogen often may lead to increased algal growth in the test waters, phosphorus should be considered the most economical key to controlling future eutrophication in the proposed cooling lake or in the source waters for the lake.

4. The data from experiments repeated during four quarters of an annual cycle suggest that evaporation more than two-fold may lead to a significant reduction in algal production, rather than an increase as might have been predicted for evaporative concentration of nutrients.

5. It was concluded that the procedure, as applied in this study should be useful in understanding the long-range effects, if any, of power plant operation. The technique is simple. Equipment and the required laboratory facilities are uncomplicated and economically modest. (Conclusions-Author).

0040

Goldman, J. C. 1976. Effects of temperature on growth constants of Selenastrum capricornutum. J. Water Pollut. Control Fed. 48(9):2215-2216.

temperature, algae, Selenastrum capricornutum, growth rates, ammonia, algal assay.

The report is a discussion of the basis behind discrepancies between two studies of growth constants and growth rates as functions of temperature for the alga Selenastrum capricornutum. One study was conducted by the author, Goldman et al. and the other study was conducted by Reynolds et al. (Abstract-Leischman-EPA).

0041

Goldman, J. C., W. J. Oswald, and D. Jenkins. 1974. The kinetics of inorganic carbon limited algal growth. J. Water Pollut. Control Fed. 46(3): 554-574.

carbon, inorganics, kinetics, pH, continuous flow, algae, Selenastrum capricornutum, Scenedesmus quadricauda, algal assay.

A series of continuous culture studies under inorganic carbon limiting conditions with axenic cultures of two representative green algae were performed in the pH range 7.05 to 7.61. Kinetic coefficients were determined for both algae. It was found that the inorganic carbon limited specific growth rates of both species were described by the Monod model when bicarbonate alkalinity was the sole inorganic carbon source. It was also found that inorganic carbon limited algal growth is a function of the total inorganic carbon present. Half-saturation coefficients for each alga were found to be low in relation to the quantities of inorganic carbon available from bicarbonate alkalinity in most natural waters. It was found that these coefficients increased significantly, however, with small increases in pH. (Abstract-JWPCF).

0042

Goldman, C. R., M. G. Tunzi, and R. Armstrong. 1969. Carbon-14 uptake as a sensitive measure of the growth of algal cultures, p. 158-170. In E. J. Middlebrooks, T. E. Maloney, C. F. Powers, and L. M. Kaack [eds], Proc. Eutroph. Bioassess. Workshop, 19-21 June 1969, U.S. Pacific Northwest Water Laboratory, Corvallis, Oregon.

carbon-14, algal assay, algae, *Chlorella pyrenoidosa*, *Selenastrum gracile*, wastewater, limiting nutrients.

The report is a summarization of results from experiments designed to test the reliability of the carbon-14 uptake method as a measurement of algal production. The method was then compared to other methods of measuring algal growth such as cell counts, absorbance as cells multiply, and determination of biomass as cells are produced. The algal species used were *Chlorella pyrenoidosa* and *Selenastrum gracile*. A series of experiments were conducted using filtered effluent from Reno-Sparks Sanitary Treatment Plant and Upper Truckee Trout Creek as additions to filtered Lake Tahoe water. Each method, cell counts, optical density, and carbon-14 uptake were used as measurements of algal growth. The author found that the *Chlorella* experiments provided a good comparison of the methods. The growth of the *Chlorella* culture was measured and expressed adequately by using the carbon-14 method. The author concluded that the carbon-14 method was easy to use for measuring algal growth within nutrient-rich and nutrient-poor waters. The carbon-14 method experiments could detect growth in waters with natural phytoplankton when the other methods gave no indication of change in biomass. The author also found that the carbon-14 method yielded values proportional to the mass of cells in the cultures. (Abstract-Leischman-EPA).

0043

Greene, J. C., W. E. Miller, T. Shiroyama, and T. E. Maloney. 1975. Utilization of algal assays to assess the effects of municipal, industrial and agricultural wastewater effluents upon phytoplankton production in the Snake River System. Water Air Soil Pollut. 4:415-434.

algal assay, rivers, algae, *Selenastrum capricornutum*, wastewater, toxicity, nitrogen, phosphorus, inhibition, bottle test.

The most characteristic water quality problem of the Snake River Basin is the excessive aquatic growth and thick blooms of algae. The cause of these aquatic growths is related to the high concentrations of basic nutrients - nitrogen and phosphorus - in the Snake River system. Industrial wastes, natural phosphate levels, irrigation return flows, municipal wastes, and the decay of aquatic biota all contribute to the nutrient balance which stimulates aquatic growths.

Algal assays were conducted on waters of eighteen Snake River and tributary sites to: (1) determine if algal growth was consistent with results predicted from review of chemical analysis for orthophosphorus and total soluble inorganic N; (2) determine if algal yields were limited to P, N, or some other nutrient essential to algal growth; and (3) predict the effects of N or P additions in algal productivity. (Abstract-Author).

0044

Greene, J. C., W. E. Miller, T. Shiroyama, and E. Merwin. 1975. Toxicity of zinc to the green alga Selenastrum capricornutum as a function of phosphorus or ionic strength, p. 28-43. In Proc. Biostim. Nutr. Assessmt. Workshop, 16-17 October 1973, U.S. Environmental Protection Agency, Corvallis, Oregon. EPA-660/3-75-034.

algal assay, algae, Selenastrum capricornutum, toxicity, zinc, phosphorus, inhibition, heavy metals, bottle test.

Experiments are described in which a modifiction of the algal assay procedure bottle test was used to evaluate Selenastrum capricornutum as a bioassay for zinc toxicity and to determine whether relationship exists between zinc toxicity and phosphorus concentrations that would affect the 14-day maximum growth yield of this alga in a defined inorganic medium. Results indicated that phosphorus (as orthophosphorus) concentrations of 0.047 to 0.930 mg/l did not significantly affect the toxicity of zinc to this alga and that algal cell numbers did not significantly affect the concentration of zinc required to produce 95% inhibition of the 14-day maximum yield. Ionic strength (specific conductance) was the dominant factor regulating zinc toxicity as the sensitivity of the test alga to zinc was inversely proportional to the increase in ionic strength of the test substrates. The factor of 2.72 plus or minus 20% multiplied by the ionic strength (micromhos/cm) of a test substrate will indicate the level of zinc (in microgram/l) that would inhibit 95% growth of the alga provided other antagonistic or synergistic constituents are absent. (Abstract-Auen-Wisconsin WRSIC).

0044-a

Greene, J. C., W. E. Miller, T. Shiroyama, R. A. Soltero, and K. Putnam. 1976. Use of algal assays to assess the effects of municipal and smelter wastes upon phytoplankton production, p. 327-335. In Proc. Symp. Terr. Aquat. Ecol. Studies of N.W., EWSC Press, Cheney, Wash.

algal assay, bottle test, algae, heavy metals, lake waters, wastewater, rivers, zinc, water quality, toxicity, *Selenastrum capricornutum*.

The Algal Assay Procedure Bottle Test is used as a tool for defining and understanding water quality management problems including: (1) the effects of municipal and industrial waste discharges upon algal productivity; (2) the effects of removing inhibitory or toxic substances from wastewaters on algal productivity when nutrients are not controlled; and (3) the degree to which wastes must be controlled to enhance water quality. The algal assay procedure is currently being used to determine the combined effects of smelter waste high in zinc content and domestic waste inflow from the City of Spokane, Washington, upon algal growth in the Spokane River basin. Long Lake, an impoundment of the Spokane River, is highly eutrophic as a result of nutrient input from the city's primary sewage treatment plant. Throughout the study Long Lake was limited for optimal algal growth primarily by either nitrogen or phosphorus. Continued presence of heavy metals in the system was indicated by increased yields of *Selenastrum capricornutum* in test waters spiked with ethylenediaminetetraacetic acid (EDTA). For approximately 80 years, heavy metal inflow throughout the system has probably affected species composition but not productivity in the natural environment. (Abstract-Author).

0045

Greene, J. C., W. E. Miller, T. Shiroyama, R. A. Soltero, and K. Putnam. 1978. Use of laboratory cultures of *Selenastrum*, *Anabaena* and the indigenous isolate *Sphaerocystis* to predict effects of nutrient and zinc interactions upon phytoplankton growth in Long Lake, Washington. Mitt. Int. Ver. Limnol. 21:372-384.

water quality, algal assay, zinc, nitrogen, phosphorus, algae, *Anabaena flos-aquae*, *Selenastrum capricornutum*, *Sphaerocystis schroeteri*, phytoplankton, bottle test, wastewater, rivers, lake waters.

The Algal Assay Procedures Bottle Test is a tool to define and understand water quality management problems including: (1) the effects of municipal and industrial waste discharges upon algal productivity; (2) the effects of removing inhibitory or toxic substances from wastewaters on algal productivity when nutrients are not controlled; and (3) the degree to which wastes must be controlled to enhance water quality. The algal assay procedure is currently being used to determine the combined effects of smelter waste high in zinc content and domestic waste inflow from the City of Spokane, Washington, upon algal growth in the Spokane River basin. Long Lake, an impoundment of the Spokane River, is highly eutrophic as a result of nutrient input from the city's primary sewage treatment plant. Throughout the study, Long Lake was limited for optimal algal growth primarily by either phosphorus or nitrogen. Continued presence of heavy metals in the system was indicated by increased yields of Selenastrum and Anabaena in test waters spiked with ethylenedinitrilotetracetic acid (EDTA); its addition was not required to support optimum growth of Sphaerocystis. For approximately 80 years, heavy metal inflow throughout the system has probably affected species composition but not productivity in the natural environment. (Abstract-Author).

0046

Greene, J. C., R. A. Soltero, W. E. Miller, A. F. Gasperino, and T. Shiroyama. 1976. The relationship of laboratory algal assays to measurements of indigenous phytoplankton in Long Lake, Washington, p. 93-126. In E. J. Middlebrooks, D. H. Falkenborg, and T. E. Maloney [eds], Biostimulation and nutrient assessment, Ann Arbor Sci., Mich.

algal assay, lake waters, algae, Selenastrum capricornutum, indigenous phytoplankton, heavy metals, bottle test, zinc, phosphorus, nitrogen, limiting nutrients, chlorophyll, reservoirs, eutrophication.

To determine if laboratory algal assays of natural waters actually reflect environmental conditions, studies were made of Selenastrum capricornutum growth potential on autoclaved and filtered water from Long Lake, Washington. Increased algal yields in EDTA spiked test waters showed toxic levels of heavy metal(s). Maximum algal yields were more than twice the minimum biomass level indicating highly eutrophic water. Samples were primarily phosphorus-limited, but sometimes the reservoir was limited so closely by both nitrogen and phosphorus that the primary limiting nutrient could not be determined. Samples collected after an underflow destroyed thermal and chemical stratification and induced extensive vertical mixing would not support algal growth in control or EDTA spiked samples. Indigenous phytoplankton contained approximately half the normal chlorophyll concentration / unit volume. Correlation was high between indigenous phytoplankton and S. capricornutum dry weight if consideration was given to whether the reservoir was stratified or homothermal. An equation was developed to predict mean indigenous phytoplankton standing crops. If the reservoir is homothermal, the equation is useful to determine approximate indigenous phytoplankton standing crop at the time of sampling. If stratified, it indicates the phytoplankton standing crop which was present approximately two weeks earlier. Another equation was developed to predict chlorophyll concentrations. (Abstract-Buchanan-Davidson-Wisconsin WRSIC).

0047

Haaland, P. T., and G. Knutson. 1973. Growth experiments with Selenastrum capricornutum Printz, p. 69-72. In Algal assays in water pollution research, Proc. Nordic Symp., 25-26 October 1972, Oslo, Norway. NORDFORSK, Secretariat of Environmental Sciences.

algal assay, temperature, light, phosphate, algae, Selenastrum capricornutum Printz, growth physiology.

The report is of a study initiated for reasons of acquiring knowledge about the growth physiology of the alga Selenastrum and to gain experience so as to design and improve general, applicable tests; also, to search for extra and possibly more suitable test algae. Growth as a function of temperature and light were measured; investigations of growth in dilute medium and growth in phosphate deficient conditions were discussed.

The authors concluded that use of Selenastrum in an algal test gave reliable results within 20 hours when increased cell numbers were indications of growth. (Abstract-Leischman-EPA).

0048

Hall, R. H. 1973. An algal toxicity test used in the safety assessment of detergent compounds. Presented at 36th Annual ASLO meeting, 12 June 1973, Salt Lake City, Utah.

algal assay, bottle test, algae, Selenastrum capricornutum, Navicula seminulum, Microcystis aeruginosa, toxicity, algistatic, detergents, algicidal, inhibition, diatoms.

Detergent components which can enter surface waters are screened for their environmental safety. An important part of this effort involves exposure of aquatic organisms to selected levels of these compounds. A standard algal toxicity test for use in this assessment is not now available. A procedure for the determination of the relative toxicity of detergent components to phytoplankton has proven useful and informative. A five-day exposure of

Selenastrum capricornutum, Microcystis aeruginosa, and Navicula seminulum to levels of test materials ranging from 0.1 to 1000 mg/l is followed by a nine-day recovery period in the absence of the compound. In vivo fluorescence measurements and cell population counts, made during the 14 days, allow quantitation of inhibition ranging from reduced growth to algistatic to algicidal responses. The 5-day Minimum Algistatic Concentration (MAC-5 day) of a detergent component is considered as potentially harmful to the phytoplankton community. An important part of the test interpretation involves data evaluation in light of potential and actual detergent component levels in surface waters. Test results on specific surfactants and builders are presented which illustrate the usefulness of these procedures in predicting the environmental safety of detergent components. (Abstract-Author).

0049

Hendricks, A. C. 1978. Response of Selenastrum capricornutum to zinc sulfides. J. Water Pollut. Control Fed. 50(1):163-168.

algae, Selenastrum capricornutum, continuous flow, algal assay, toxicity, heavy metals, zinc, sulfides, oxygen.

Oxygen production by Selenastrum capricornutum was determined after exposing the cells to various concentrations of ionic zinc, sulfide, and zinc plus sulfide. One mg/l and 2.5 mg/l of Zn^{2+} and S^{2-} reduced oxygen production by 50 percent or more. However, the two exhibited antagonistic behavior when tested together. When 5 mg/l of Zn^{2+} was mixed with 2.5 mg/l of S^{2-} only a slight reduction in oxygen production occurred. (Abstract-JWPCF).

0050

Hostetter, H. P. 1976. Rapid bioassay for algal toxins in effluents and surface waters. Ohio State Univ. RF NO 3934, U.S. Environmental Protection Agency, Cincinnati, Ohio, Final Report. 41 p.

algal assay, algae, Selenastrum capricornutum, pollution, nutrients, toxicity, DO, oxygen electrode, limiting nutrients, copper, mercury, zinc, agricultural watersheds, heavy metals.

A preliminary technique has been developed for a rapid bioassay to measure the effects of pollutants or nutrients on the metabolism of algae. A Clark type oxygen electrode is used to measure changes in dissolved oxygen in a photosynthetic chamber containing the water to be tested and the test organism. The test alga is incubated 24 hr in the water prior to the test. Determination of photosynthetic rate takes 10-15 min. Evaluation of the water can be made the day following collection. Results of the rapid bioassay are compared with the Algal Assay Procedure Bottle Test. In culture medium the rate of photosynthesis is directly proportional to the concentration of nutrients limiting photosynthesis. Photosynthesis in waters collected from agricultural watersheds (expected to be high in nitrogen) is not enhanced or only slightly enhanced by fortification with algal growth medium or nitrate. Photosynthesis of cells incubated in waters with toxic concentrations of copper, mercury, or zinc is inhibited. (Abstract-Author).

0050-a

Ionescu, A., and L. Gavrila. 1972. Contribution to study of the influence of some stimulatory and inhibitory substances on growth and photosynthesis on algae. Stud. Cercet. Biol. Sci. Bot. 24(1):9-16. In French.

algae, inhibition, indolebutyric acid, colchicine, atrazine, chlorelline extracts, scenedesmine, diatoms, Scenedesmus, Chlorella, Selenastrum, Navicula, Cocconeis, Amphora, Cymbella, Gomphonema, algal assay.

In research designed to investigate the multiplication, growth, and intensity of photosynthesis in algae in pure or combined cultures, different doses of IAA, indolebutyric acid, colchicine, atrazine, and extracts of chlorelline and scenedesmine were administered to cultures of Scenedesmus, Chlorella, and Selenastrum. Phytoplankton (diatoms-predominantly Cocconeis, Navicula, Amphora, Cymbella, Gomphonema) were also tested. The alga cultures responded favorably to the action of the growth factors even during the first few days of application, while colchicine and extracts of old cultures slowed cellular multiplication. Photosynthesis was inhibited by colchicine and atrazine and favored by the growth substances. In stopping photosynthesis, atrazine revealed the existence of a heterotrophism in Scenedesmus that gave it the opportunity to live without chlorophyll assimilation. Also, certain combinations of culture media and growth substances removed the retarding effect on multiplication and photosynthesis in old cultures and allowed an increase in biomass. (Translated Summary-Biological Abstracts, Inc.).

0051

Jadlocki, J. F., Jr., J. Saldick, S. E. Coleridge, W. W. Smith, J. W. Brown, and C. S. Nicholson. 1976. Effects of water hardness, phosphorus concentration and sample pretreatment of the Algal Assay Procedure Bottle Test, p. 323-334. In E. J. Middlebrooks, D. H. Falkenborg, and T. E. Maloney [eds], Biostimulation and nutrient assessment, Ann Arbor Sci., Mich.

algal assay, bottle test, algae, *Selenastrum capricornutum*, phosphorus, pretreatment, lake waters, water hardness.

To determine if different types of pretreatment affect the results of the Algal Assay Procedure Bottle Test, chemical and algal assays were made on samples from lakes in New York and Indiana. Algal assays using *Selenastrum capricornutum* were done on water samples before treatment or after pretreatment by autoclaving and/or filtration. The results indicated that pretreatment of the water samples may cause erroneous results in the Algal Assay Procedure Bottle Test. The controlling factors include water hardness, phosphorus concentration, and standing algal crops. Waters which are very hard (above 200 ppm as calcium carbonate) and have a high orthophosphate concentration (above 10 micrograms/L) may form a precipitate during autoclaving which effectively depletes the available phosphorus, especially if the precipitate is removed by filtration. A substantial growth of algae (over 200 fluorescence units from *in vivo* chlorophyll *a*) in these waters would release enough dissolved phosphorus during autoclaving to cause a net increase in soluble phosphorus concentration. Much of the increased analyzable orthophosphate was apparently not available for *Selenastrum* growth. Since the expected growth corresponding to the increased orthophosphate analyses was not observed, filtration pretreatment may also remove large amounts of total phosphorus from eutrophic water samples. (Abstract-Buchanan-Davidson-Wisconsin WRSIC).

0052

Joint Industry/Government Task Force on Eutrophication. 1969. Provisional algal assay procedure. Joint Indust./Govt. Task Force Eutroph., Grand Central Station, New York. 62 p.

algal assay, algae, *Selenastrum capricornutum*, *Anabaena flos-aquae*, *Microcystis aeruginosa*, continuous flow, bottle test, nutrients, eutrophication, pollution, nitrogen fixation, carbon-14.

The procedure described was developed by a team of international experts under the sponsorship of the Joint Industry/Government Task Force on Eutrophication and represents the first step toward developing reliable and reproducible tests to determine the capacity of various aquatic environments to grow algae. It is emphasized that the procedures presented are provisional and are not standardized tests. Three procedures are described: a bottle test, a continuous-flow chemostat test, and an _in situ_ test. Detailed descriptions of equipment required, laboratory procedures, analytical techniques, and sample preparation are given. Indicator organisms selected for use in the tests are _Selenastrum capricornutum_, a green alga, _Anabaena flos-aquae_, a blue-green alga capable of fixing nitrogen; and _Microcystis aeruginosa_ a blue-green alga not capable of fixing nitrogen. (Abstract-Uttermark-Wisconsin WRSIC).

0052-a

Justice, C., S. Murray, P. S. Dixon, and J. Scherfig. 1972. Evaluation of materials for use in algal culture systems. Hydrobiol. 40(2):215-221.

bottle test, continuous flow, algal assay, algae, _Selenastrum capricornutum_.

The report is of experiments conducted to test new materials used in continuous flow algal assays. The materials tested were Pyrex and Kimax glass flasks; neoprine stoppers and Cafe-au-lait rubber stoppers. The authors concluded that new materials used in tests, as new, had some inhibitory effects on algal growth and that the new materials should be soaked prior to use. The authors support testing the new materials before incorporating the materials into important algal assays. (Abstract-Leischman-EPA).

0053

Kallqvist, T. 1973. Algal assay procedure (bottle test) at the Norwegian Institute for Water Research, p. 5-18. _In_ Algal assays in water pollution research, Proc. Nordic Symp., 25-26 October 1972, Oslo, Norway. NORDFORSK, Secretariat of Environmental Sciences.

algal assay, bottle test, limiting nutrients, algae, _Selenastrum capricornutum_, _Phaeodactylum tricornutum_, _Skeletonema costatum_, toxicity, PCB, phosphorus, wastewater, diatoms.

The report is an outlined description of the laboratory procedure using the algal assay bottle test to determine the following: (1) the toxicity of substances on growth of algae; (2) the maximum standing crop in dilute medium; (3) the effect of phosphorus on the maximum standing crop; (4) sewage effluent effects on algal growth; and (5) determination of limiting nutrients. The algal species used were *Selenastrum capricornutum*, *Skeletonema costatum* and *Phaeodactylum tricornutum*. Brief examples of results are given, graphs included, but no discussion is provided. (Abstract-Leischman-EPA).

0054

Kallqvist, T. 1973. Use of algal assay for investigating a brackish water area, p. 111-124. *In* algal assays in water pollution research, Proc. Nordic Symp., 25-26 October 1972, Oslo, Norway. NORDFORSK, Secretariat of Environmental Sciences.

wastewater, nitrogen, phosphorus, N:P ratios, rivers, limiting nutrients, algae, *Selenastrum capricornutum*, *Phaeodactylum tricornutum*, *Skeletonema costatum*, fjord, algal assay, bottle test, diatoms.

The algal assay procedure was used to investigate the Frierfjord and influences of wastewater upon the nutrient status of the fjord. The algal species used were *Selenastrum capricornutum*, *Phaeodactylum tricornutum*, *Skeletonema costatum*. The author found that the wastewater had a stimulatory effect on the growth of algae in the fjord as well as growth further out from the coastal area. Phosphate was found to be the primary limiting nutrient. A high N:P ratio was calculated for the wastewater. The author concluded that the algal assay procedure was advantageous in that it expressed clearly the characterizations of waters in terms of nutrient status more than would chemical analyses. (Abstract-Leischman-EPA).

0055

Kallqvist, T. 1974. Algal growth potential of six Norwegian waters receiving primary, secondary, and tertiary sewage effluents. Ver. Int. Assoc. Theor. Appl. Limnol. Proc. 19(3):2070-2081.

AGP, wastewater, lake waters, fjord, algae, *Selenastrum capricornutum*, *Phaeodactylum tricornutum*, phosphorus, nitrogen, limiting nutrients, algal assay, diatoms.

The experiments with algal growth in mixtures of three different kinds of treated sewage and six receiving waters have shown that the growth potential in the mixture depends on the composition of the receiving water as well as the wastewater. This is especially the case for chemically treated wastewater (CTW). With this kind of effluent, the P concentration in the receiving water is important for the resulting growth potential. In P deficient waters the fertilizing effect is very low, but in waters having P in excess as is usually the case in sea water, low concentrations of chemically treated sewage may give the same increase as biologically treated sewage (BTW). In such waters treatment methods that reduce N would be the best solution (Goldman _et al_, 1974). The reason why an addition of CTW to P deficient waters causes almost no increase in growth potential is that the P content in the sewage after the chemical precipitation is low. It must be pointed out, however, that the assays were carried out on filtered water, which means that the P adsorbed to the particulate matter was removed more efficiently than in most treatment plants. Later experiments have shown that much of this particulate-bound P is readily available to the algae and consequently that algal assays on filtered water from a chemical treatment plant give values which are too low (Lofgren, 1974). This finding emphasizes the point that filtering of the effluents from the chemical treatment plants will bring about better P removal.

Of the mechanically and biologically treated wastewaters, the MTW contains the higher level of nutrients and also gives a greater increase of the growth potential in all receiving waters. The difference between these two types of wastewater regarding their fertilizing effect is, however, small. BTW seems to have a more immediate stimulating effect on the algal growth than MTW, resulting in a higher growth rate of the test algae in cultures with BTW. The reason for this has not been investigated. (Conclusion-Author).

0056

Katko, A. 1975. Algal assays for the National Eutrophication Survey, p. 44-52. _In_ Proc. Biostim. Nutr. Assess. Workshop, 16-17 October 1973, U.S. Environmental Protection Agency, Corvallis, Oregon. EPA-660/3-75-034.

algal assay, lake waters, bottle test, algae, _Selenastrum capricornutum_, phosphate, nitrogen, wastewater.

The algal assay procedure was incorporated into the National Eutrophication Lake Survey. The investigation was of the nutrient status (primarily nutrient reduction to improve water quality) of approximately 800 lakes in the United States. The report is of the programs completed and those in progress, for all 27 states east of the Mississippi River. The programs involved 485 lakes and 2500 stream sampling sites. *Selenastrum capricornutum* was the test alga used for the algal assay. Results are given for the 1972 sampling year. A discussion is given of the algal assay as a tool; and, the advantages and disadvantages in relating the results of algal assays to real world situations in the attempt to assess and classify all the lakes within the Lake Survey. (Abstract-Leischman-EPA).

0057

Keenan, J. D., and M. T. Auer. 1974. The influence of phosphorus luxury uptake on algal bioassays. J. Water Pollut. Control Fed. 46(3):532-542.

algal assay, phosphorus, uptake, bottle test, algae, *Anabaena flos-aquae*, *Microcystis aeruginosa*, *Selenastrum capricornutum*.

The effect of luxury uptake of orthophosphate on the sensitivity of algal growth bioassays was determined. Short-term bioassays consisted of gravimetrically determined harvested dry weights; long-term bioassays consisted of manometrically determined photosynthetic rates. Phosphorus metabolism was studied by measurement of stored phosphorus, alkaline phosphatase activity, and phosphate concentration in the nutrient medium. *Anabaena flos-aquae*, *Microcystic aeruginosa*, and *Selenastrum capricornutum* exhibited luxury uptake of phosphorus. However, *M. aeruginosa* and *S. capricornutum* did not exhibit alkaline phosphatase activity. It was concluded that short-term bioassays are less sensitive to phosphorus concentration than long-term bioassays. The influence of luxury uptake is a function of the phosphorus concentration in the original growth medium and of the length of the starvation period. (Abstract-JWPCF).

0058

King, D. L., and J. T. Novak. 1974. The kinetics of inorganic carbon-limited algal growth. J. Water Pollut. Control Fed. 46(7):1812-1816.

inorganics, carbon, algae, Selenastrum capricornutum, Scenedesmus quadricauda, growth rates, algal assay.

The data of an earlier paper by J. C. Goldman and others is reinterpreted to show that Selenastrum capricornutum and Scenedesmus quadricauda both respond in a kinetic fashion to free CO_2 concentration of water for their photosynthetic C source, and that their specific growth rate is not related directly to total inorganic C in the water as suggested by Goldman et al. (Abstract-Biological Abstracts, Inc.).

0059

Klotz, R. L., J. R. Cain, and F. R. Trainor. 1975. A sensitive algal assay: An improved method for analysis of freshwaters. J. Phycol. 11(4):411-414.

algal assay, algae, Selenastrum capricornutum Printz, rivers, bottle test.

An algal assay capable of detecting the carrying capacity of nutrient-poor rivers was developed. In order to eliminate carry-over of nutrients, thus masking the true nutrient condition of the water tested, a new growth medium was developed. This maintenance medium contained less than 4 $mg \cdot l^{-1}$ total inorganic salts. With an inoculum of 10^4 cells ml^{-1} the test organism Selenastrum capricornutum Printz was grown in sterile-filtered river water and transferred daily with a 1:1 dilution. The assay was carried out for 4 or 5 days, at which time cell number was calculated, and the doublings per day were determined. Comparisons are made with similar but less sensitive assays, on the basis of both daily and 5-day readings. (Summary-Author).

0059-a

Kotai, J., T. Krogh, and O. M. Skulberg. 1978. The fertility of some Norwegian inland waters assayed by algal cultures. Mitt. Int. Ver. Limnol. 21:413-436.

eutrophication, lake waters, rivers, nutrients, bottle test, algal assay, algae, Selenastrum capricornutum Printz, nitrogen, phosphorus, AGP.

In an investigation and comparative study of more than 400 lakes and rivers in East Norway, algal assays were conducted to determine algal growth potential values; the limiting nutrients; and, the fertility of the waters examined. The algal assays were found to provide more information about biostimulatory and bioinhibiting properties of the waters than the chemical analyses. The authors felt that both chemical analysis and algal assays should be combined in studies of polluted waters. (Abstract-Leischmn-EPA).

0060

Kotai, J., T. Krogh, K. Ormerod, and O. M. Skulberg. 1976. Estimation of algal growth potential. NIVA Publ., D2-25, Oslo, Norway. 32 p.

AGP, algae, Selenastrum capricornutum, Phaeodactylum tricornutum, algal assay, diatoms.

The report is a detailed, outlined procedure for use and application of the algal growth potential, AGP, as a method to test a laboratory sample under set standard conditions, either freshwater or marine water. The authors provide detailed descriptions of the necessary reagents, apparatus, test organisms, and methods to conduct an experiment utilizing the AGP method. The test organisms suggested are Selenastrum capricornutum and Phaeodactylum tricornutum. (Abstract-Leischman-EPA).

0060-a

Laake, M. 1978. Monitoring the effects of chemical and biological waste water treatment in situ by dialysis cultures of freshwater algae. Mitt. Int. Ver. Limnol. 21:453-472.

continuous flow, algal assay, algae, dialysis, chlorophyll a, growth kinetics, ATP, heavy metals, zinc, copper, lead, cadmium, Scenedesmus quadricauda, Selenastrum capricornutum, Chlamydomonas reinhardtii, Asterionella formosa, diatoms.

Unialgal and mixed cultures of freshwater algae were inoculated in 12 dialysis bags (32 ml) attached to a rotating wheel submerged in throughflow boxes of white polyethylene. The surface was freely exposed to daylight. Two parallels were harvested every third or fourth day and the growth followed by measuring optical density at 430 mm. The cells were analyzed for organic carbon, chlorophyll a and phaeophytin, ATP and the heavy metals Cu, Zn, Cd and Pb. Four experiments each with four types of treated waste water at 0.5 and 5 vol % concentrations were conducted: secondary Al-treatment + biological oxidation pond (Al + BD), secondary Al-treatment (Al), activated sludge biological treatment + Al-treatment (B + Al) and activated sludge with simultaneous Al-treatment (SIM). Two experiments with *Scenedesmus quadricauda* and two starting with equal numbers of *Scenedesmus*, *Selenastrum capricornutum* *Chlamydomonas reinhardtii* and *Asterionella formosa* were performed.

The results show that SIM generally gave highest cell yield, while the others were more or less equal. With mixed cultures the ATP content per cell decreased with decreasing waste water load, but increased with *Scenedesmus*. Mean levels of 0.6 µg ATP/mg C confirm phosphate limitation. Chlorophyll content significantly decreased with decreasing load, while no differences between treatments were evident. *Chlamydomonas* and *Selenastrum* did grow to a lesser extent at 5% than at 0.5% dosing, while *Asterionella* diminished almost completely. All mixed cultures were dominated by *Scenedesmus*, which appeared to grow better than in pure culture.

The yield factors to phosphate and the maintenance rates were calculated from growth rate in linear phase and asymptotic biomass, respectively. These factors gave the best basis for comparative evaluation of the treatment processes. The results show that the algal growth efficiency on phosphate limitation may be ranged in the order Al + BD, Al, B + Al and SIM, thus indicating the degree of environmental stress in the cultures. The efficiency also decreased with increasing load for all treatments.

From data on heavy metal content concentration factors in the order of magnitude 10^6 for Cd, $4 \cdot 10^5$ for Pb and $2.5 \cdot 10^4$ for Zn were calculated. High levels were correlated with high maintenance rates versus phosphate. (Summary-Author).

0060-b

Lambou, V. W., L. R. Williams, S. C. Hern, R. W. Thomas, and J. D. Bliss. 1976. Prediction of phytoplankton productivity in lakes, p. 696-700. *In* Proc. Conf. Environ. Modeling and Simulation, U.S. Environmental Protection Agency, 19-22 April 1976, Cincinnati, Ohio. EPA-600/9-76-016.

algal assay, bottle test, algae, chlorophyll *a*, water quality, phytoplankton, lake waters, limiting nutrients, phosphorus, nitrogen, N:P ratios, *Selenastrum capricornutum*, *Anabaena*, *Microcystis*.

This study presents relationships between phytoplankton productivity as measured by yearly mean chlorophyll *a* levels, and ambient water quality and hydrologic measurements. Among the nutrients examined, phosphorus forms were most highly correlated with chlorophyll a levels. The effects of such factors as retention time, primary nutrient limitation, stratification, and macrophyte dominance upon productivity responses are evaluated. Additional parameters related to productivity include turbidity, Secchi disc, nitrogen to phosphorus ratio, pH, total alkalinity, and forms of inorganic nitrogen. Discussions of the factors affecting phytoplankton productivity and the application of the limiting nutrient concept are included. (Summary-Author).

0061

Lange, W. 1971. Limiting nutrient elements in filtered Lake Erie water. Water Res. 5(1):1031-1048.

algal assay, lake waters, limiting nutrients, nitrate, phosphorus, cobalt, iron, algae, Anabaena circinalis, Microcystis aeruginosa, Nostoc muscorum, Selenastrum capricornutum, bottle test.

Biweekly laboratory bioassays were made using filtered Lake Erie waters from the Bass Island region to simulate the effect of an influx of individual nutrients to Lake Erie water. Each sample during the 1969 growing season was inoculated singly with each of three blue-green algae and one green algal species. Portions of each inoculated water sample were enriched with one of 16 essential nutrient elements and cell numbers were determined in the fourth week after exposure under controlled culture conditions. Comparative cell counts showed nitrate-N was the nutrient the most frequently required and was limiting for these algal species in about 2/3 of the water samples. Phosphorus, cobalt and chelated iron were limiting for about 1/3 of their cultures. To achieve maximum growth stimulation generally required a combination of several nutrient elements. An Aphanizomenon bloom occurred naturally in Lake Erie at the water sampling site and after its collapse from nutrient insufficiency, the water samples were found to be toxic to certain of the test algal species. (Abstract-Author).

0062

Lee, C. C., R. F. Harris, J. K. Syers, and D. E. Armstrong. 1971. Adenosine triphosphate content of Selenastrum capricornutum. Appl. Microbiol. 21(5):957-958.

ATP, adenosine triphosphate, phosphorus, algal assay, algae, Selenastrum capricornutum.

The adenosine triphosphate content of Selenastrum capricornutum was maintained within 1.4 to 3.4 µg of adenosine triphosphate/mg (dry weight) of living biomass at different growth stages in media of different phosphorus concentrations. (Abstract-Author).

0063

Lehmusluoto, P. O. 1973. Algal assay procedure in use in Finland, p. 33-34. In Algal assays in water pollution research, Proc. Nordic Symp., 25-26 October 1972, Oslo, Norway. NORDFORSK, Secretariat of Environmental Sciences.

algal assay, bottle test, nitrogen, phosphorus, algae, Ankistrodesmus falcatus, Selenastrum capricornutum, Chlorella sp., wastewater.

The report is a description of an algal assay procedure, the AGP (Algal Growth Potential), used in Finland to investigate the nutrient status of various types of waters. The test organisms described as those being used are Ankistrodesmus falcatus, Selenastrum capricornutum, and Chlorella sp. Brief explanations of steps in the procedure and references are given. (Abstract-Leischman-EPA).

0064

Lindemann, E. G., and G. P. Volpp. 1971. Carbon dioxide and trace elements as factors in algal assay procedures. Presented Water, Air, and Waste Chem., Amer. Chem. Soc., Washington, D.C. 12 p.

algal assay, bottle test, carbon dioxide, algae, Selenastrum capricornutum, trace elements.

The report is a discussion of the investigation on the effect of bacteria-generated carbon dioxide on the growth of the green alga Selenastrum capricornutum and the final yield of the algae under laboratory conditions. The second portion of the report covers the importance of trace elements and the required precautions in identifying them as limiting nutrients. The authors found that the maximum cell count was more than three times greater in the flasks supplied with bacteria-generated CO_2 as opposed to the flasks with CO_2 supplied from the atmosphere. The authors suggested the use of polycarbonate flasks for the procedure sensitive to trace elements and the technique described in the report. (Abstract-Leischman-EPA).

0065

Lindmark, G. 1973. Bioassay with Selenastrum capricornutum to assess the nutrient status of lakes and the fertilizing influence of interstitial water, p. 73-80. In Algal assays in water pollution research, Proc. Nordic Symp., 25-26 October 1972, Oslo, Norway. NORDFORSK, Secretariat of Environmental Sciences.

lake waters, algal assay, algae, Selenastrum capricornutum, wastewater, eutrophication, restoration.

The algal assay method, as a form of investigation of the nutrient status of lake waters, was incorporated into the Lake Trummen Restoration Project. The study used the algal species Selenastrum capricornutum. The restoration process was by removal of lake sediments. The algal assay was used as a tool to study the nutrient status of interstitial waters and lake waters before, during, and after restoration. The author found a marked seasonal variation in the nutrient levels; phosphorus was always in excess: additions of the interstitial water to the lake increased heavy algal growth. (Abstract-Leischman-EPA).

0065-a

Little, L. W., and M. A. Chillingworth. 1974. Effect of 56 selected dyes on growth of the green alga Selenastrum capricornutum. Chap. 2. In Dyes and the environment - Reports on selected dyes and their effects, v. II, Amer. Dye Manuf. Inst., Inc., New York.

dyes, toxicity, inhibition, algal assay, algae, Selenastrum capricornutum, fish, Pimephales promelas, bottle test.

Fifty-six commercially important dyes, selected by American Dye Manufacturers Institute Ecology Committee, were tested for toxicity to the green alga Selenastrum capricornutum. The Algal Assay Procedure developed by the National Eutrophication Research Program--Environmental Protection Agency was employed. Concentrations of 1 and 10 mg/l of dye were assayed. Growth of algae was measured by direct counts at 7 and 14 days of incubation.

In general, basic dyes, even at a concentration of 1 mg/l, inhibited algal growth to less than 1% of that attained in the dye-free controls. Dyes of other classes were generally less toxic. Overall, the effect of dyes on algal growth correlates well with that of the same dyes on the fathead minnow, Pimephales promelas. (Abstract-Author).

0066

Little, L., and D. Pittman. 1974. Determination of herbicides and their relationships to algal growth. Proc. Nebr. Acad. Sci. Affil. Soc. 84:48.

herbicides, dacthal, aldrin, algal blooms, lake waters, algae, Selenastrum, phytoplankton, Anacystis, Pediastrum, algal assay.

Herbicide concentrations, with emphasis on dacthal, and their relationships to algal growth was studied in connection to algal blooms. Dacthal was examined as a possible stimulant. Anacystis was stimulated by a concentration of 0.01 ppm dacthal, but Pediastrum and Selenastrum results were inconclusive. Algae, plankton, water, and soil samples from Bluestem Lake near Lincoln, Nebraska were found to contain pesticides, tentatively aldrin and dacthal. Extraction, cleanup, and analysis methods for lake samples outlined. Lake algal blooms are not, conclusively, due to the stimulating effects of herbicides. (Abstract Only-Author).

0066-a

Long, E. T., and G. D. Cooke. 1978. Phosphorus variability in three streams during storm events: chemical analysis vs. algal assay. Mitt. Int. Ver. Limnol. 21:441-452.

eutrophication, streams, phosphorus, lake waters, algal assay, bottle test, algae, Selenastrum capricornutum, urban runoff, forest runoff.

The majority of calculations of P loading to lakes have been made on the basis of a chemical analysis. The present study was conducted to evaluate and compare chemically determined total phosphorus (TP) and soluble reactive phosphorus (SRP) to biologically available phosphorus (AP) loading of lakes during hydrological events (storm runoff) and calendar events (weekly samples).

Three streams draining a forest, an urban region, and a lake, all located in the Twin Lakes Watershed (NE Ohio, U.S.A.) were studied. Water samples were collected throughout the course of storm events. They were also collected at weekly intervals for a 51 day period prior to storm sampling. Measurement of stream flow, suspended solids, soluble reactive and total P, amount of precipitation (rain), and storm intensity were taken. A portion of each water sample was assayed for biologically available P using a modified Algal Assay Procedure with Selenastrum capricornutum Printz.

Changes in loading of the three forms of P, calculated from weekly sampling, are depicted.

The response of the urban and forest area to two storm events was one of increased water loading. The response of the lake outflow to storm initiation was increased water loading, but this stream did not display the abrupt amplitude changes that were exhibited by the other two streams. Changes in suspended solids loading during storm events is depicted. The changes in TP, SRP and AP loadings are also illustrated.

Comparison of loading rates from calendar events to those from hydrological events showed that considerably greater amounts of all three forms of P were contributed during storm runoff from all three areas. The urban area, however, contributed substantially larger amounts of all three P forms than did the other two areas.

The conclusions reached from this study were that short-term phenomena, such as storm runoff, do play a major role in P loading to lakes. It was also shown that land use activities greatly affect these loading rates and that urban areas have the greatest impact. Available phosphorus (AP) loadings were considerably different than total phosphorus (TP) loading, both during storm events and during base flow. During base flow, only a small fraction of the P income was AP, this fraction increased greatly during storm flow from the urban and forest areas, but not from lake discharge. The suggestion is made that the use of biologically available P in nutrient budgets might greatly enhance the predictability of limnological models relating change in P income to the degree of eutrophication. (Modified Summary-Author).

0067

Maki, A. W., L. D. Geissel, and H. E. Johnson. 1975. Toxicity of the lampricide 3-trifluoromethyl-4-nitrophenol to 10 species of algae, p. 3-17. In U.S. Fish Wildl. Serv. Invest. Fish. Control 56.

algae, Scenedesmus quadricauda, Stigeoclonium tenue, Selenastrum capricornutum, Chlorella pyrenoidosa, Cylindrospermum sp., Anabaena flos-aquae, Anabaena cylindrica, Nostoc linokia, Nitzschia sp., Navicula pelliculosa, growth inhibition, TFM, algal assay, diatoms.

The toxicity of analytical and field grades of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) to unialgal cultures of four green algae, four blue-green algae, and two species of diatoms was examined in 96-h toxicity tests. Growth was measured by daily optical density readings, cell counts of nonfilamentous species, and a gravimetric determination of maximum standing crop at the end of the tests. A 50% inhibition of growth (calculated from optical density data) occurred at concentrations less than 10 mg/l of TFM for all species tested except Chlorella pyrenoidosa, which was resistant at concentrations greater than 15 mg/l. Inhibition of growth was lowest in the blue-green species (50% effect levels, 9-10 mg/l), generally intermediate in the green algae, and severe in diatoms (50% effect levels, 1-4 mg/l). Field grade TFM tended to be more toxic than the analytical grade. Similar toxicity relationships were indicated by each of the three types of measurements made.

Growth tests conducted with high concentrations of TFM and subsequent filtration of the cells and resuspension in toxicant-free medium indicated that exposure to TFM at concentrations of 30 mg/l for 96 h did not destroy the viability of algal cells but temporarily inhibited growth. (Abstract-Author).

0068

Malone, R. F., K. A. Voos, W. J. Grenney, and J. H. Reynolds. 1976. The effects of media modifications upon Selenastrum capricornutum in batch cultures, p. 267-292. In E. J. Middlebrooks, D. H. Falkenborg, and T. E. Maloney [eds.], Biostimulation and nutrient assessment, Ann Arbor Sci., Mich.

nitrogen, limiting nutrients, carbon, phosphate, buffers, algal assay, continuous flow, algae, Selenastrum capricornutum, mathematical model.

A project was conducted in order to develop a mathematical model that predicted growth of the alga Selenastrum capricornutum in nitrogen limited conditions. The experiments and observations led to a further investigation of a medium that suited the requirements for calibration. The previous experiments resulted in some atypical growth. Investigations were of the use of sodium bicarbonate as a carbon source; the use of a phosphate buffer to adjust the pH; and, the nitrogen limitation in relation to nitrogen concentrations within the medium. The authors found that the use of sodium or potassium phosphate buffer on the medium resulted in a reduction of algal growth by removing an essential nutrient, which their data confirmed. It was also suggested that a toxic effect might be reducing growth but experiments did not indicate so. Sodium bicarbonate was found to be an adequate source of a carbon supplement but in high concentrations would effect growth by altering pH. It was suggested that a buffer be used in conjunction with sodium bicarbonate. (Abstract-Leischman-EPA).

0069

Maloney, T. E., and W. E. Miller. 1975. Algal assays: Development and application, p. 344-355. _In_ Water quality parameters, Amer. Soc. Test. Mater., ASTM STP 573.

water quality, limiting nutrients, algal assay, bottle test, wastewater, lake waters, streams, algae, _Selenastrum capricornutum_.

The development, refinement, standardization, and practical application of an algal procedure are described. Results of experiments are presented to illustrate the use of the algal assay procedure to determine algal growth-limiting nutrients in fresh waters, including lakes and streams; for the assessment of receiving waters to determine their nutrient status and sensitivity to change; for the assessment of the effects of changes in waste treatment processes on receiving waters; and for the evaluation of materials and products to determine their potential effects on algal growth. (Abstract-Author).

0070

Maloney, T. E., W. E. Miller, and N. L. Blind. 1973. Use of algal assays in studying eutrophication problems, p. 205-214. _In_ S. H. Jenkins [ed.], Advances in water pollution research, Sixth Internat. Conf., Jerusalem, Pergamon Press, Oxford and New York.

algal assay, eutrophication, wastewater, lake waters, phosphorus, nitrogen, nutrients, limiting nutrients, algae, _Selenastrum capricornutum_.

The use of algal assays to assist in the solution of practical eutrophication is illustrated by the studies described herein. We have demonstrated their use in assessing the effects of changes in waste treatement processes on receiving waters, in identifying algal growth-limiting nutrients, and in assessing receiving waters to determine their nutrient status and sensitivity to change. We feel that algal assays will also be a valuable asset in dealing with other practical problems such as evaluating materials and products to determine their potential effects on algal growth in receiving waters.

The algal assay cannot, of course, be used by itself in evaluating and solving eutrophication problems. If combined, however, with good chemical and physical analyses as well as thorough limnological investigations, it can be an extremely useful procedure. (Summary-Author).

0071

Maloney, T. E., W. E. Miller, and T. Shiroyama. 1972. Algal responses to nutrient additions in natural waters: I. Laboratory Assays, p. 134-140. In G. F. Likens [ed.], Nutrients and eutrophication: The limiting nutrient controversy, Spec. Symp. Amer. Soc. Limnol. Oceanogr., Vol 1, Allen Press, Kansas.

lake waters, nitrogen, phosphorus, carbon, algae, Selenastrum capricornutum Printz, algal assay, bottle test.

Laboratory algal assays were conducted on waters from nine Oregon lakes of varying water quality. Selenastrum capricornutum Printz was used as the test species in an algal assay using the Provisional Algal Assay Procedure (Joint Industry/Government Task Force on Eutrophication 1969). Additions of nitrogen, phosphorus, and carbon, singly and in combination, were made to the waters and algal growth rates were determined. The addition of phosphorus alone greatly stimulated algal growth rates in four of the waters and the addition of nitrogen alone slightly stimulated algal growth rates in waters of two of the lakes. Three of the waters were capable of supporting relatively high algal growth rates without nutrient additions, and in one highly oligotrophic lake water the addition of nitrogen, phosphorus, and carbon had no effect on algal growth rates. In all cases algal growth rates were directly proportional to the amounts of dissolved phosphorus in the waters, but there was no obvious correlation between algal growth rates and concentrations of nitrogen and carbon. (Abstract-Author).

0072

McDonald, G. C., and N. L. Clesceri. 1973. Effect of wastewater organic fractions on the growth of selected algae, p. 479-496. In E. Glass [ed.], Bioassay techniques and environmental chemistry, Ann Arbor Sci., Mich.

wastewater, algal assay, carbon, phosphorus, organic fractions, bottle test, algae, Anabaena flos-aquae, Selenastrum capricornutum.

The existence and extent of algal growth enhancement brought about by the addition of wastewater organic fractions to representative algal cultures were determined. A sample of effluent from a conventional activated sludge facility located at Batavia, New York was subjected to fractionation using gel chromatographic techniques. Subsequent to filtration and freeze drying, the concentrated wastewater was separated into organic fractions by gel chromatography. Algae selected for the study were Selenastrum capricornutum and Anabaena flos-aquae. Growth of both species was determined by absorbance measurements at 750 nm with a Beckman DU-2 Spectrophotometer. Organic compounds contained in wastewater fractions have been found to exert a growth-enhancing effect on the two algae species. The exact nature of the causative growth factors and the pathway by which they achieve their effects are as yet unknown, and further investigative efforts are being conducted to assess generality of these results. This undertaking is important as increased algal productivity may continue to occur if such factors are general constituents of wastewater effluents, notwithstanding the removal of nitrogen and phosphorus from such effluents. (Abstract-Jones-Wisconsin WRSIC).

0073

McDonald, G. C., R. D. Spear, P. J. Lavin, and N. L. Clesceri. 1970. Kinetics of algal growth in austere media, p. 97-105. In J. E. Zajic [ed.], Properties and products of algae symposium, Plenum Publ. Corp., New York.

nutrients, nitrogen, phosphorus, limiting nutrients, lake waters, inhibition, growth kinetics, algae, Selenastrum capricornutum, media, algal assay.

The report is of experiments conducted to determine the effects of different concentrations of nitrogen and phosphorus on growth of the alga Selenastrum capricornutum. Initially the use of the two media, modified Gorham's medium and Basic ASM medium, diluted with glass filtered water, was part one of the experiments. Secondly, the media were diluted with filtered Lake George waters. Varied concentrations of nitrogen and phosphorus were added and growth of the algae was measured. The authors found that the reduction of nitrogen concentrations was less effective on reduction of algal growth than the reduction of phosphorus concentrations for both experiments. The effect of nitrogen reduction in the Lake George:media mixture was more pronounced than in the distilled water:media mixture. The alga was found to be phosphorus limited; Lake George waters used as the dilutent had an inhibitory effect on growth other than the limiting nutrients; and, phosphorus limitation was found to be the least pronounced in the Lake George:Basic ASM medium mixture. (Abstract-Leischman-EPA).

0074

Middlebrooks, E. J., D. H. Falkenborg, and T. E. Maloney, editors. 1975. Biostimulation and nutrient assessment. Ann Arbor Sci., 391 p.

algal assay, nutrients, eutrophication, toxicity, limiting nutrients, wastewater, sediments, phosphorus, lake waters, bottle test, growth rates, indigenous phytoplankton, algae, Selenastrum capricornutum, Anabaena flos-aquae, continuous flow, water hardness.

The application of the Algal Assay Procedure Bottle Test and its modifications are discussed in relation to various ecological and environmental variables. Contributions focus on the application of the test in biostimulation and toxicity testing; field evaluation of the test on surface waters of North Carolina, its application to define the effects of wastewater effluents upon algal growth in multiple use river systems; and the relationship of laboratory algal assays to measurement of indigenous phytoplankton in Long Lake, Washington. The test's applicability to compare algal growth responses of Selenastrum capricornutum and Anabaena flos-aquae in waters collected from Shagawa Lake, Minnesota, seasonal variation of algal biomass production potential and nutrient limitation in Yaquina Bay, Oregon; and algal nutritional bioassays of Lake Wylie, North Carolina, is described. Nutrient assessment as a basis for lake management priorities, as applied to Wisconsin lakes, is reviewed. Other contributions compare semi-continuous and continuous flow bioassays; the effects of media modifications upon S. capricornutum in batch cultures; sediment-water microcosms designed for assessment of nutrient interactions; effects of water hardness, phosphorus concentration and sample pretreatment on the test; and an elutriate method to determine the significance of dredged material disposal in open waters. (Abstract-Auen-Wisconsin).

0075

Middlebrooks, E. J., E. A. Pearson, M. Tunzi, A. Adinarayana, P. H. McGauhey, and G. A. Rohlich. 1971. Eutrophication of surface water: Lake Tahoe. J. Water Pollut. Control Fed. 43(2):242-251.

ammonia, carbon, eutrophication, lake waters, nitrogen, nitrogen oxide, phosphorus, nutrients, streams, bottle test, algae, *Selenastrum gracile*, continuous flow, algal assay.

Although nitrogen and phosphorus are major factors in the nutrient-rich or eutrophic condition that leads to algal blooms, their presence is not always the critical factor in eutrophication. The authors make an effort to develop suitable methods of assaying the algal growth-stimulating potential of various influents to lakes and streams and to apply these methods to an evaluation of their ability to increase the eutrophication of water. A test algae, *Selenastrum gracile*, was used to assess quantitatively biostimulation by exposure to surface runoff, seepages, effluents from various wastewater treatment processes, and Lake Tahoe (Calif.) water. Initial growth response to the test algae was much greater with batch-type assay data than with steady-state conditions. Both batch and continuous flow assays indicated that the biostimulatory properties of secondary effluents were much greater than that from raw or primary treated wastes. Wastewater effluents of all types showed higher growth response rates than could be accounted for by the amount of N and P present in the samples. (Abstract-JWPCF).

0076

Miller, W. E., and T. E. Maloney. 1971. Effects of secondary and tertiary wastewater effluents on algal growth in a lake-river system. J. Water Pollut. Control Fed. 43(12):2361-2365.

algal assay, eutrophication, nitrogen, nutrients, phosphorus, water quality, wastewater, lake waters, rivers, algae, *Selenastrum capricornutum*, bottle test.

The biostimulatory effects of secondary and tertiary treated wastewater effluent were determined on the Shagawa Lake-Burntside River system in Minnesota. Chemical characteristics of the river were consistent year-round and supported growth of test algae in concentration of 5 X 10^3 cells/ml. The addition of 0.02 mg phosphorus (P)/l increased algal growth. The addition of 1.0 mg nitrogen (N)/l resulted in no increase. The addition of 2 percent secondary effluent increased algal growth significantly. Ten percent tertiary effluent did not stimulate growth. Lake water supported algal growth, and correlation was shown between algal growth responses and N and P content of lake water samples. The lake was N and/or P deficient, depending on the time of the year. (Abstract-JWPCF).

0077

Miller, W. E., J. C. Greene, and T. Shiroyama. 1976. Application of algal assays to define the effect of wastewater effluents upon algal growth in multiple use river systems, p. 77-92. In E. J. Middlebrooks, D. H. Falkenborg, and T. E. Maloney [eds.], Biostimulation and nutrient assessment, Ann Arbor Sci., Mich.

wastewater, rivers, limiting nutrients, nitrogen, phosphorus, trace elements, heavy metals, toxicity, N:P ratios, algal assay, bottle test, algae, *Selenastrum capricornutum*.

The report is of application of algal assays to define effects of domestic and industrial waste discharges on algal growth in the Spokane River System, Washington. The design and interpretation of laboratory algal assays is discussed. Algal responses to nitrogen and phosphorus limitations, trace element deficiency, and heavy metal toxicity were determined. The critical nitrogen:phosphorus ratio to support *Selenastrum capricornutum* was 11.3:1 micrograms. This ratio can assess algal growth limitations in natural waters. Waters with N:P ratios less than 10 are considered nitrogen-limiting while waters with N:P ratios above 10 may be phosphorus-limiting for algal growth. Correlation of algal assays with yields predicted from N:P ratios depend on the reliability of test procedure. Total soluble inorganic nitrogen (nitrite + nitrate + ammonia) and orthophosphate concentrations or availability in samples were calculated by dividing maximum assay yields by the total soluble inorganic nitrogen or orthophosphate yield coefficient. That samples do not attain the predicted assay yield or nutrient limitation status may be due to growth-limiting nutrients other than nitrogen or phosphorus, presence of toxic materials, or unreliable chemical determinations of orthophosphate and total soluble inorganic nitrogen. Addition of EDTA before assay can help determine nutrient enrichment problems in river systems receiving heavy metal wastes. (Abstract-Buchanan-Davidson-Wisconsin WRSIC).

0078

Miller, W. E., J. C. Greene, and T. Shiroyama. 1976. Use of algal assays to define trace-element limitation and heavy metal toxicity, p. 317-325. In Proc. Symp. Terr. Aquat. Ecol. Studies of N.W., EWSC Press, Cheney, Wash.

heavy metals, trace elements, algal assay, bottle test, zinc, copper, cadmium, inhibition, algae, Selenastrum capricornutum.

Recent attention has focused on the biological response to conditions of trace-element deficiency and heavy metal stress. This approach is necessitated, in part, by the fact that metals are bonded by ligands and particles in varying degrees. Therefore, absolute metal concentrations contained in the wastewater effluent or receiving water do not necessarily reflect the degree to which they affect biological organisms. The Algal Assay Procedure Bottle Test has been used to define the toxicity of zinc, copper and cadmium under controlled laboratory conditions. Those results indicate that the sensitivity of Selenastrum capricornutum to these metals is inversely proportional to the ionic strength of the test substrate. Other factors affecting toxicity, such as increasing metal concentration, contact time and environmental test characteristics, were also evaluated. Inhibition by zinc, 0-100 percent, was linear with increasing concentration while increasing the concentration of both copper and cadmium resulted in linear inhibition up to 20 and 40 percent, respectively. Length of contact time, up to 21 days, reversed inhibition of growth of the test organism when exposed to sublethal concentrations of copper, cadmium, and zinc. (Abstract-Author).

0079

Miller, W. E., J. C. Greene, and T. Shiroyama. 1978. The Selenastrum capricornutum Printz Algal Assay Bottle Test: Experimental design, application, and data interpretation protocol. U.S. Environmental Protection Agency, Corvallis, Oregon. EPA-600/9-78-018. 125 p.

algal assay, bottle test, wastewater, nutrients, inhibition, heavy metals, lake waters, rivers, data interpretation, experimental design, trace elements, pretreatment, toxicity, algae, Selenastrum capricornutum Printz.

This document is the product of intensive research to improve and expand the understanding of results obtained from the Algal Assay Procedure Bottle Test (USEPA, 1971) to enable investigators to define the stimulatory and/or inhibitory interaction(s) of municipal, industrial and agricultural wastes upon algal productivity in natural waters.

This research was designed to determine:

(1) The impact of nutrients and/or changes in their loading upon algal productivity;

(2) Whether the growth response of *Selenastrum capricornutum* reflects the response of indigenous species;
(3) The feasibility of the assay test protocol to evaluate heavy metals;
(4) The capability of the assay to define the effect(s) of complex wastes; and
(5) If the assay information can be applied to define and assist in the management of real-world situations. (Preface-Author).

0080

Miller, W. E., T. E. Maloney, and J. C. Greene. 1974. Algal productivity in 49 lake waters as determined by algal assays. Water Res. 8(9):667-679.

lake waters, algal assay, phosphorus, nitrogen, limiting nutrients, algae, *Selenastrum capricornutum*.

Algal assays were conducted on waters from 49 American lakes to determine whether phosphorus, nitrogen, or some other nutrient was the limiting factor upon algal productivity.

A comparison of the known trophic state of 23 American lakes and their bioassay response is presented. Phosphorus limited algal growth in 35 of the 49 lakes assayed. Nitrogen was limiting in eight lakes and constituents other than nitrogen and phosphorus were limiting to six lakes. The results of this study indicated phosphorus limitation decreased as the productivity of the lake waters increased. (Abstract-Author).

0080-a

Miller, W. E., J. C. Greene, E. A. Merwin, and T. Shiroyama. 1978. Algal bioassay techniques for pollution evaluation, p. 9-16. *In* Toxic materials in the aquatic environment. Seminar Oregon St. Univ., Water Res. Res. Inst., Spring 1978, Corvallis, SEMIN WR 024-78.

toxicity, algal assay, bottle test, eutrophication, water quality, heavy metals, limiting nutrients, nitrogen, phosphorus, zinc, wastewater, stimulation, inhibition, lake waters, impoundment, algae, *Selenastrum capricornutum*.

The paper is a discussion of the utilization of the Algal Assay Bottle Test procedure in studying nutrient limitation, heavy metal toxicity and in evaluating complex wastes. The test organism is the alga *Selenastrum capricornutum*. The basic techniques involved in the procedure are included in the discussion of the development and application of the AA:BT procedure. (Abstract-Leischman-EPA).

0081

Miller, W. E., J. C. Greene, T. Shiroyama, and E. Merwin. 1975. The use of algal assays to determine effects of waste discharge in the Spokane River system, p. 113-131. *In* Proc. Biostim. Nutr. Assess. Workshop, 16-17 October 1973. U.S. Environmental Protection Agency, Corvallis, Oregon. EPA-660/3-75-034.

wastewater, heavy metals, zinc, toxicity, inhibition, limiting nutrients, nitrogen, phosphorus, bottle test, algal assay, algae, *Selenastrum capricornutum*.

This paper is a report on the assessment of waters impacted by industrial and domestic waste effluents and their effect on algal growth. The area of investigation was the Spokane River system, Idaho and Washington. Algal assays were performed on samples collected at seven sites along the Spokane River system. The assays were conducted to determine the nutrient status of the waters (nitrogen and phosphorus concentrations); the critical nutrient responsible for algal growth in the river system; and, the level of zinc that would prevent algal growth (algistatic level). The authors found that in sampled areas of the river system where heavy metals, particularly zinc, were not dominant, additions of nitrogen and phosphorus stimulated algal growth; however, the process reversed in waters below the locations of smelter and mining industries, growth decreasing proportionally to increase in zinc concentrations. The method used to remove zinc from the sample waters and the addition of a chelator (EDTA) both resulted in increased algal growth with the nutrient additions. The algal assay was found to be effective in assessing the problem of nutrient enriched waters containing heavy metals. (Abstract-Leischman-EPA).

0082

Mitchell, D. 1973. Algal bioassays for estimating the effect of added materials upon planktonic algae in surface waters, p. 153-158. *In* E. Glass [ed.], Bioassay techniques and environmental chemistry, Ann Arbor Sci., Mich.

algal assay, bottle test, PAAP, MAAP, NTA, trisodium nitrilotriacetate, diversity indices, algae, *Selenastrum capricornutum*, *Anabaena flos-aquae*, *Microcystis aeruginosa*.

The Provisional Algal Assay Procedure (PAAP) Bottle Test and the Microcosm Algal Assay Procedure (MAAP) were compared. The PAAP was found to be an excellent analytical tool for assessing the physiological effects of various materials upon algae, and for determining the limiting nutrient in water samples. However it lacks the capability, which the MAAP does have, of delineating interactions between the ecosystem and the material being assessed, as well as between the various algal species making up the natural community. Examination of sodium nitrilotriacetate (NTA) showed no significant effects even at 200 mg/liter (at least 1000 times the expected environmental levels) in the PAAP. Likewise, NTA showed no effects in the MAAP at the lower levels, but at 200 mg/liter a stimulation of algal growth and loss of diversity was observed. (Abstract-Author).

0083

Monahan, T. J. 1976. Lead inhibition of chlorophycean microalgae. J. Phycol. 12(3):358-362.

lead, phosphate, pH, toxicity, algae, *Scenedesmus* sp., *Scenedesmus obtusiusculus*, *Selenastrum capricornutum*, *Chlorella pyrenoidosa*, *Chlorella ellipsoidea* *Chlorella vulgaris*, *Ankistrodesmus* sp., algal assay.

The effects of Pb on the growth of 9 microalgae representing 4 chlorophycean genera were investigated in axenic laboratory cultures. Variations in sensitivity to Pb existed among the algae tested with availability of phosphate often limiting growth. Under conditions in which phosphate was growth limiting at pH 6.2 *Chlorella pyrenoidosa* Chick, *C*. *ellipsoidea* Gernick, *C* *vulgaris* Beij. *Scenedesmus* sp. (IUCC 1591), *S* *obtusiusculus* Chodat and *Selenastrum capricornutum* Printz first showed toxicity at 0.5 mg l^{-1} Pb. Two planktonic *Scenedesmus* spp. and an *Ankistrodesmus* sp. were more tolerant, developing toxicity at 1 mg l^{-1} Pb. The relationship of phosphate supply to Pb toxicity was investigated with *Selenastrum capricornutum* and *S*. *obtusiusculus*. At pH 8.0, *Selenastrum* developed toxicity at 3 mg l^{-1} Pb either with or without phosphate. When phosphate supply was growth-limiting, 6 times as much Pb (i.e. 3 vs 0.5 mg l^{-1}) was required to demonstrate toxicity with *Selenastrum* at pH 8.0 as opposed to pH 6.2. *S*. *obtusiusculus* was inhibited at 0.5 mgl^{-1} Pb in phosphate-limiting medium and developed toxicity at 1 mg · l^{-1} in media containing phosphate. Control of colonial morphology with *S*. *obtus-*

iusculus was inhibited. Phosphate availability and pH seem to be important factors determining Pb toxicity with some algae. (Summary-Author).

0084

Morton, S. D., and T. H. Lee. 1974. Algal blooms: possible effects of iron. Environ. Sci. Technol. 8(7):673-674.

iron, manganese, phosphorus, algae, Chlorella pyrenoidosa, Dictyosphaerium pullchellum, Selenastrum capricornutum, Anabaena circinalis, Gloeotrichia echinulata, Microcystis aeruginosa, algal assay.

Iron, in concentrations of 0.1-1.0 mg/l, causes a shift in the dominant type of algae grown in batch cultures from relatively unobjectionable greens to the scum-forming blue-greens, without causing a significant change in the total algal biomass. Results were not dependent on the manganese concentration (0-0.40 mg/l) or the phosphorus concentration (0.07-7.0 mg/l). (Abstract-Author).

0085

Murray, S., J. Scherfig, and P. Dixon. 1971. Evaluation of algal assay procedures: PAAP batch test. J. Water Pollut. Control Fed. 43(10):1991-2003.

algal assay, trace elements, nutrients, light intensity, hydrogen, carbon dioxide, eutrophication, bottle test, algae, Selenastrum capricornutum.

Preliminary investigations have demonstrated four important difficulties involved in the Provisional Algal Assay Procedure (PAAP) Batch Test. The four difficulties were: (1) the effects of medium preparation on the final composition of the medium; (2) pH changes during the test period in relation to carbon dioxide availability; (3) the method of gas addition in the batch test units; and (4) the effects of light intensity on algal growth. Because of these limitations, certain modifications to the standardized procedures were developed. A new culture media preparation which eliminates the precipitation of essential trace elements was developed and provides a reliable reference medium for algal assay work. The addition of CO_2-enriched air reduced the growth-limiting effects of CO_2 and stabilizes the pH at 7.5 to 8.0. Aeration was shown to be more effective than ventilation for CO_2 addition and gaseous as a result of bicarbonate utilization. Light intensities of 500 ft-c reduced growth rates of *Selenastrum capricornutum* in bottle test experimentation compared with 350 ft-c. (Abstract-Lowry-Texas WRSIC).

0085-a

Natarajan, K. V. 1960. Mineral nutrition of *Selenastrum westii*, p. 156-161. *In* P. Kachroo [ed.], Symposium of Algology, Indian Council Agric. Research, New Delhi.

algal assay, algae, media, nutrients, nitrogen, phosphorus, N:P ratios, *Kirchneriella subsolitaria*, *Selenastrum westii*.

The present observations showed that in a purely inorganic medium of suitable composition, *Selenastrum westii* showed rapid growth and gave high final yields of cells. It was observed that while the alga required about 20.4 ppm N for its maximum growth, the phosphate concentration required was only 0.9 ppm P. The nitrogen:phosphorus ratio of 22.6:1 found for this alga is in close agreement with the values found for other algae (Harvey, 1940; Miller and Fogg, 1957). *Kirchneriella subsolitaria*, another member belonging to the same family was found to require an N:P ratio of approximately 15:1 (Potash, 1958). The minimum concentrations of the essential major elements for the production of maximum numbers of cells of this alga were 20.4 ppm N, Ca and 2.5 ppm Fe. The growth of the alga in this solution was found to be 135, 27.36 of that in the basic solution. (Abstract-Biological Abstracts, Inc.).

NOTE: The above article was not located, as there were errors in translation of the title of book and the source. The National Agricultural Library, Dictionary Catalog of N.A.L., Vol. 59, U.S.D.A., does list the book under the editor's name.

0086

Nyholm, N. 1977. Kinetics of phosphate limited algal growth. Biotech. Bioengr. 19:467-492.

growth kinetics, limiting nutrients, phosphate, continuous flow, phosphorus, Michaelis-Menten equation, chlorophyll *a*, algae, *Chlorella pyrenoidosa*, *Selenastrum capricornutum*, algal assay.

The kinetics of phosphate limited growth of two green algae *Chlorella pyrenoidosa* and *Selenastrum capricornutum* have been studied in chemostats.

Several kinetic models which express the specific growth rate as a function of the intracellular phosphorus content have been examined, and one of the models was found to be significantly better than the other models. The principles of this model were described in a recent paper by Nyholm.

The kinetics of phosphate uptake have been investigated by adding pulses of phosphate to the chemostats. The uptake by phosphorus deficient cells could be described by Michaelis-Menten kinetics for phosphate concentrations below approximately 500 μg P/liter. Further, with the assumption of a discontinuous adjustment of the uptake rate at the onset of phosphorus deficiency, a complete kinetic model for growth and phosphate removal is proposed.

The mean cell size and the contents of chlorophyll a and RNA per unit dry weight have been measured for C. pyrenoidosa as a function of the dilution rate. (Summary-Author).

0086-a

Olofsson, J. A., Jr., and F. E. Woodard. 1977. Effects of pH and inorganic carbon concentrations upon competition between Anabaena flos-aquae and Selenastrum capricornutum. Land and Water Res. Inst., Univ. Maine, Orono, Completion report.

inorganic carbon, competition, inhibition, pH, photosynthetic rates, uptake, eutrophication, algal blooms, algae, Anabaena flos-aquae, Selenastrum capricornutum, bottle test, algal assay.

Underlying reasons for seasonal dominance of lacustrine phytoplankton by the blue-green algae have not heretofore been precisely defined. Relative competitiveness of a selected green-alga and blue-green alga was examined in relation to culture pH and total inorganic carbon (C_T) in an effort to uncover a mechanism which might be influential in blue-green algal bloom initiation. Short-term growth experiments were conducted employing Anabaena flos-aquae, a bloom forming blue-green alga and Selenastrum capricornutum, a green alga. A self-contained polarographic dissolved oxygen electrode system was employed to measure net photosynthetic oxygen evolution and respiratory oxygen consumption at 25°C.

Media containing total inorganic carbon levels of 0.107, 0.214, 0.429 and 1.072 mg l^{-1} were employed at pH levels of 7.0, 8.0, 9.0 and 10.0. Algal cells of uniform culture history were harvested in mid- to late log phase growth. They were washed and resuspended in the test medium and then placed in the dissolved oxygen probe cell. Dissolved oxygen changes during light and dark periods permitted calculation of oxygen evolution rates per unit mass of algal material in the test suspension. The chosen C_T levels imposed carbon limitation upon the algal suspensions as evidenced by increasing photosynthesis as C_T increased at given pH levels. All other nutrients were present in excess.

A. flos-aquae, at a given level of C_T, exhibited increasing photosynthetic rate as pH increased and reached a maximum in the region of pH 9.5 to 10.0. On the other hand, at given C_T, levels, S. capricornutum exhibited decreasing photosynthetic rates as pH was increased. Photosynthetic rates of A. flos-aquae exceeded those of S. capricornutum above pH 7.5, indicating potential pH-related enhancement of blue-green photosynthesis.

Carbonic anhydrase was implicated in facilitating photosynthetic rates in A. flos-aquae by using a specific inhibitor of carbonic anhydrase activity. Outside the pH range of 8.35 to 9.0, when the inhibitor Diamox (5-acetamido-1,3,4-thiadiazole-2-sulphomanide) was introduced, significantly reduced photosynthetic rates were observed in A. flos-aquae. It was concluded that A. flos-aquae has a higher affinity for inorganic carbon (as evidenced by photosynthetic rates) than does S. capricornutum due to carbonic anhydrase activity. Based upon the observation that Diamox had no significant effect upon photosynthetic oxygen evolution within the pH range of 8.35 and 9.0, it was concluded that carbonic anhydrase is not important here. Also, A. flos-aquae may preferentially utilize HCO_3^--carbon. The maximum kinetic rate of HCO_3^- availability via carbonate alkalinity equilibria was equal to the maximum rate of carbon fixation estimated from oxygen evolution rates.

The overall conclusion drawn from this investigation is that periodic dominance of the lacustrine phytoplankton by bloom forming blue-green algae, as represented by A. flos-aquae, may be related to competitive uptake of inorganic carbon facilitated by carbonic anhydrase activity. Facilitated carbon transport in blue-green algae may provide additional insight into the management of blue-green algal blooms in surface water resources. (Abstract-Author).

0087

Oswald, W. J., and S. A. Gaonkar. 1969. Batch assays for determination of algal growth potential, p. 23-38. In E. J. Middlebrooks, T. E. Maloney, C. F. Powers, and L. M. Kaack [eds.], Proc. Eutroph. Biostim. Assessmt. Workshop, 19-21 June 1969, Berkeley, Calif. SERL and NERP sponsored.

algal assay, bottle test, growth rates, kinetics, trace elements, toxicity, eutrophication, AGP, algae, *Selenastrum capricornutum*, *Anabaena flos-aquae*, *Microcystis aeruginosa*, *Chlorella pyrenoidosa*, *Scenedesmus obliquus*, *Euglena gracilis*.

Successful use of algal cultures in studies of algal growth potentials, trace mineral analyses, tastes and odors, and toxicity, gives reason to believe they may be valuable for assessment of eutrophication potential in the "Bottle Test" version of the Provisional Algal Assay Procedure (PAAP), as proposed by the National Eutrophication Research Program. While tests may lack elegance, they are simple, flexible, versatile, reliable, and applicable--criteria established as desirable for that program. Author feels that certain characteristics of the test, as presently recommended, require alteration. It is doubtful, for example, if requirements for vigorous shaking, rigid temperature control, and accessory gases are necessary. In addition, simplified incubation techniques and apparatus should be evaluated. Several of the techniques proposed for assessment of growth in cultures may be unnecessarily complex or expensive, whereas techniques of fluorescence analysis and volumetric analysis of growth--not now being considered--should be studied. Parameters of growth in PAAP could be evaluated in terms of growth levels for which they may be most useful; thus, a rational system might involve enumeration of cells for algal growth potentials (in milligrams/liter) of 0.1-1.0; fluorescence, for 10-100; and gravimetry, for 100-1000. (Abstract-Eichhorn-Wisconsin WRSIC).

0088

Palmer, M. 1969. A composite rating of algae tolerating organic pollution. J. Phycol. 5(1):78-82.

pollution, organics, algae.

From information on pollution-tolerant algae compiled from reports from 165 authors, the genera and species most often referred to as significant fall into a relatively stable series. Diatoms, pigmented flagellates, green, and blue-green algae are all well represented among the pollution-tolerant genera and species. The top 8 genera are *Euglena*, *Oscillatoria*, *Chlamydomonas*, *Scenedesmus*, *Chlorella*, *Nitzschia*, *Navicula*, and *Stigeoclonium*, and the top 5 species, *Euglena viridis*, *Nitzschia palea*, *Oscillatoria limosa*, *Scenedesmus quadricauda*, and *Oscillatoria tenuis*. In some genera, e.g., Euglena, a single species is far more significant than all others as a pollution-tolerant form. In other genera, e.g., Oscillatoria, only a slight difference distinguishes the polluton tolerance of 2 or more species. Algal genus and species pollution indices are presented for use in rating water samples with high organic pollution. (Summary-Author).

0089

Paoletti, C., B. Pushparaj, G. Florenzano, P. Capella, and G. Lercker. 1976. Unsaponifiable matter of green and blue-green algal lipids as a factor of biochemical differentiation of their biomasses: I. Total unsaponifiable and hydrocarbon fraction. Lipids 11(4):258-265.

lipids, hydrocarbons, sterols, phytoplankton, *Spirulina platensis*, *Spirulina* sp., *Calothrix* sp., *Nostoc commune*, *Scenedesmus quadricauda*, *Chlorella* sp., *Uronema gigas*, *Uronema terrestre*, *Selenastrum gracile*, carotenoids, unsaponifiables, algae, algal assay.

As part of a program to study the chemical composition of algal biomasses, the composition of the unsaponifiable matter of the lipids of ten algal species (five *Myxophyceae* and five *Chlorophyceae*) was investigated. The total unsaponifiable content, its general composition, and the components of the hydrocarbon fraction are discussed in the present paper. The unsaponifiable content of green algae is constantly higher than that of the blue-green ones, with the exception of *Chlorella*. In both algal classes, the major components are hydrocarbons and sterols. Blue-green algae are richer in hydrocarbons, whereas the green ones contain higher amounts of sterols. In most of the species examined, at least 48 components are present in the hydrocarbon fraction. Each algal species shows a characteristic gas liquid chromatography pattern, but n-C_{17} is always one of the most abundant components. Generally, the procaryotic blue-green algae show a simpler hydrocarbon composition than the eucaryotic green algae, which contain higher amounts of high mol. wt. components. Unsaturated hydrocarbons are generally present in very limited quantities, with the exception of Spirulina sp., and Chlorella sp., which contain a C_{17} alkene. Green algae also contain appreciable amounts of a C_{27} monoene and of squalene. (Abstract-Author).

0090

Parker, M. 1977. The use of algal bioassays to predict the short and long term changes in algal standing crop which result from altered phosphorus and nitrogen loadings. Water Res. 11(8):719-725.

algal assay, bottle test, lake waters, phosphorus, nitrogen, mathematical model, algae, Selenastrum capricornutum, standing crop.

Bioassays were performed on filtered water from Jackson Lake, Wyoming, using the Algal Assay Procedure-Bottle Test with Selenastrum capricornutum as the assay organism. It is hypothesized that (i) short- and long-term additions of phosphorus (P) and nitrogen (N) to waters should be evaluated separately, and (ii) only bioassay treatments of lake water plus phosphorus plus nitrogen (LW + P + N) should be used to predict the effect of long-term phosphorus additions on algal standing crop. Equations are developed directly from the corresponding bioassay responses to predict short-term increases in the lake's algal standing crop. Accepting the hypothesis, an equation describing the response of S. capricornutum to LW + P + N treatments is derived to predict the long-term effects of phosphorus additions on algal standing crop. This equation is then combined with another derived to describe the steady state concentration of phosphorus in lake water. The resultant equation predicts the percent change in algal standing crop as a function of any change in the lake's phosphorus loading. In most cases, use of LW + P rather than LW + P + N treatments to predict changes in algal standing crop will lead to quite different interpretation of bioassay data and probably to different management decisions. (Abstract-Author).

0091

Parr, M. P., and R. V. Smith. 1976. The identification of phosphorus as a growth-limiting nutrient in Lough Neagh, using bioassays. Water Res. 10(12):1151-1154.

algal assay, lake waters, limiting nutrients, nitrogen, phosphorus, wastewater, bottle test, algae, Selenastrum capricornutum, phytoplankton, Aphanizomenon flos-aquae, Oscillatoria redekei.

Algae were used in monthly bioassays of Lough Neagh water to evaluate nutrient limitation. Blue-green algae were limited by chelated iron; but once this limitation was overcome both nitrogen and phosphorus were necessary to produce a large growth response. The green alga *Selenastrum* showed a low requirement for chelated iron. Addition of phosphorus was necessary in all bioassays for prolonged algal growth, confirming the strategy of removing phosphorus from sewage effluents to reduce algal levels in the Lough. (Abstract-Author).

0092

Parra, B. O. 1973. Qualitative study of phytoplankton of Laguna Verde, Concepcion (Chile), excluding diatoms. Gayana Bot. 24:1-26. In Spanish.

phytoplankton, algae, classification.

Thirteen phytoplankton samples collected in Laguna Verde, Concepcion, Chile, were studied. 35 species, 2 forms and 2 varieties of Fungi, Cyanophyta, Chrysophyceae and Chlorophyta were found. Four genera, sixteen species and two varieties are new records for the microflora of Chile. These taxa are *Chlorobotrys regularis*, *Pleodorina californica*, *Polyedriopsis spinulosa*, *Selenastrum gracile*, *Chodatella longiseta*, *Chodatella quadriseta*, *Franceia droescheri*, *Franceia ovalis*, *Scenedesmus dimorphus*, *Scenedesmus ellipsoideus* *Scenedesmus opoliensis*, *Scenedesmus spinosus*, *Arthrodesmus octocornis*, *Staurastrum irregulare*, *Staurastrum iversenii* var. *americanum*, *Staurastrum polymorphum*, *Staurastrum tetracerum* var. *biverruciferum*. (Translated Abstract-Author).

0093

Payne, A. G. 1973. Environmental testing of citrate bioassays for algal stimulation. Proc. 16th Conf. Great Lakes Res. 16:100-115.

algal assay, stimulation, growth rates, sodium citrate, bottle test, wastewater, lake waters, inhibition, eutrophication, algae, *Selenastrum capricornutum*, *Microcystis aeruginosa*.

Possible effects of sodium citrate on eutrophication were investigated throughout a growing season using the bioassay procedure of the Algal Assay Procedure Bottle Test. Primary and secondary treated sewage, with and without citrate, was added to filtered waters from 17 locations across the U.S., including Lake Erie and Lake Michigan. The algae selected for study were bacteria-associated Selenastrum capricornutum and Microcystis aeruginosa; growth was followed by cell counts.

Sewage generally stimulated algal growth to higher maximum standing crops than the control. Citrate added to sewage normally had no additional effect on algal maximum standing crops and no stimulation by addition of citrate alone was detected in any water. Citrate added to sewage prevented die-off of Microcystis in four waters and citrate added without sewage treatment and at relatively high levels of 1 and 10 mg/l caused some growth inhibition of Selenastrum in seven waters. In 156 test situations, citrate added to sewage caused an increase in growth rate in eight situations and a decrease in growth rates in six situations. In most instances, the different growth rates caused no differences in maximum standing crops. (Abstract Only-Author).

0094

Payne, A. G. 1975. Responses of the three test algae of the Algal Assay Procedure Bottle Test. Water Res. 9(4):439-445.

algal assay, bottle test, light intensity, nutrients, phosphorus, wastewater, limiting nutrients, algae, Selenastrum capricornutum, Microcystis aeruginosa, Anabaena flos-aquae.

The Algal Assay Procedure (AAP) Bottle Test utilized three test organisms which, under standardized culture conditions, give a range of responses to the physical and nutritional states of test waters. The test algae of the AAP are Selenastrum capricornutum, Microcystis aeruginosa and Anabaena flos-aquae. The responses of these organisms to light intensities and to micro- and macronutrients are described. Phosphate spikes as low as 1-2 μg P l^{-1} are shown to give a measurable bioassay response in highly oligotrophic waters.

The AAP test algae are used to assess the effects of nutrient enrichment with sewage in waters from a cross-section of U.S. lakes. Enrichment of oligotrophic waters with primary or secondary sewage effluent results in significant growth of the three algae, while sewage additions to eutrophic waters cause little or no significant increase in algal growth rate or maximum standing crop. The response of the AAP algae also indicate that nutrient removal by tertiary treatment greatly reduces the stimulatory properties of sewage. In the presence of tertiary sewage, growth rate and maximum standing crop of the three test organisms are near those of lake water controls.

The AAP test protocol is shown to be useful in the determination of limiting nutrients and in the comparison of the algal growth potential of natural waters to that of defined media. (Abstract-Author).

0095

Payne, A. G. 1976. Application of the algal assay procedure in biostimulation and toxicity testing, p. 3-28. *In* E. J. Middlebrooks, D. H. Falkenborg, and T. E. Maloney [eds.], Biostimulation and nutrient assessment, Ann Arbor Sci., Mich.

algal assay, bottle test, lake waters, wastewater, eutrophication, phosphorus, chlorophyll *a*, trace elements, chelation, heavy metals, toxicity, *Microcystis aeruginosa*, *Anabaena* sp., *Navicula seminulum*, phytoplankton, inhibition, algistatic, algicidal, algae, *Selenastrum capricornutum*, diatoms.

The algal assay procedure, used in conjunction with physical/chemical measurements of natural waters has been successfully applied to the assessment of the algal growth potential of those waters.

In testing nutrient conditions of natural waters, the AAP showed logical differences among waters and by seasonal patterns. Samples from oligotrophic waters, when filtered, inoculated and maintained under laboratory culture conditions, supported low standing crops of algae throughout the year. Natural populations in these waters also remained low throughout the year. Eutrophic waters during the winter months were rich in nutrients and supported high maximum standing crops of test algae in AAP tests. The following spring, these lakes, as predicted, supported high natural population levels.

The AAP can provide useful information on the relative chelating capacity of synthetic compounds, sewage effluent, and natural chelators in the aquatic environment. The sensitivity of laboratory-cultured *Microcystis aeruginosa* to low levels of free metals makes this a useful test organism for trace metal/chelator studies. The level of Zn in AAP media is close to the toxicity level for *Microcystis* and may need to be lowered for optimization of *Microcystis* growth.

The application of the AAP has been expanded to include an assessment of algal toxicity of test materials. A toxicity test is described which is both operationally simple and environmentally meaningful. The Minimum Algistatic

Concentration after 5 days represents that level of a test material which causes no net change in the population of the test algae after chronic exposure. Inclusion of a recovery period following this contact allows interpretation of the MAC-5 day to be the level of the test material which causes primary inhibitory effects at the cellular level but does not cause permanent or irreversible damage to the test culture. (Summary-Author).

0096

Payne, A. G., and R. H. Hall. 1978. Application of algal assays in the environmental evaluation of new detergent materials. Mitt. Int. Ver. Limnol. 21:507-520.

algal assay, bottle test, detergents, phosphate, wastewater, chelation, trace elements, toxicity, algae, Selenastrum capricornutum, Microcystis aeruginosa, Anabaena flos-aquae, stimulation.

Algal assays have been used extensively in the environmental safety evaluations of detergent candidate materials. The Algal Assay Procedure Bottle Test (AAP), developed in cooperation with the U.S. Environmental Protection Agency, has been successfully applied, with modifications, to studies of stimulation, chelation, and toxicity effects.

Algal stimulation studies have been conducted on materials considered for use as detergent phosphate substitutes. These materials have been tested at realistic and exaggerated environmental concentrations, alone and in the presence of sewage, in surface waters from across the U.S. which differed in hardness and varied from infertile to highly fertile.

The AAP has been used to develop information on the relative chelating ability of detergent builder candidates in defined systems. A comparative chelating capacity test has been developed by altering the trace metal/ chelator system of AAP medium. Results of this test demonstrate the ability of specific chelators to protect against metal toxicity and give some measure of effects of detergent builders on the aquatic environment.

The AAP has been further modified to provide an assessment of algal toxicity of detergent surfactants. A dose/response relationship can be obtained which quantifies the effects of concentrations of test materials on net cell production. The test is both operationally simple and environmentally meaningful. (Abstract-Author).

0097

Payne, A. G., and R. H. Hall. 1978. A method for measuring algal toxicity and its application to the safety assessment of new chemicals. Proc. Sec. Symp. Aquat. Tox., 31 October and 1 November 1977, Cleveland, Ohio. ASTM publ. In press.

algal assay, bottle test, toxicity, algistatic, algicidal, trace elements, inhibition, algae, _Selenastrum capricornutum_, _Microcystis aeruginosa_, _Navicula seminulum_, _Dunaliella tertiolecta_, diatoms.

An algal toxicity test has been developed specifically for use in the environmental safety assessment of new chemicals. The test is based on the Algal Assay Procedure Bottle Test, but has been modified in order to make the procedure useful for toxicity assessment. A five-day exposure of freshwater or marine unialgal cultures to a range of test material concentrations is followed by a nine-day recovery period in the absence of the test material. Cell counts or _in vivo_ fluorescence measurements made during the study allow quantification of toxic responses ranging from reduced growth to algistatic to algicidal. The primary toxicity effect sought in this method is the algistatic response, which is both easily determined and environmentally meaningful. The algistatic concentration of a test material, as derived by this method, is the concentration that causes no net change in cell number after chronic exposure but permits regrowth when the cells are resuspended in fresh media without the test material. The environmental significance of this type of measurement relative to other measurements of algal toxicity is discussed. (Abstract-Author).

0098

Plumb, R. H., Jr. 1975. A bioassay dilution technique to assess the significance of dredged material disposal, p. 335-346. _In_ E. J. Middlebrooks, D. H. Falkenborg, and T. E. Maloney [eds.], Biostimulation and nutrient assessment, Ann Arbor Sci., Mich.

nutrients, nitrogen, phosphorus, sediments, interstitial waters, algal assay, bottle test, dredging, algae, _Selenastrum capricornutum_, _Dunaliella tertiolecta_.

A potential problem at open-water dredged material disposal sites is that nutrients (nitrogen and phophorus) associated with dredged sediment and interstitial water may stimulate algal growth. Conventional bioassays are not applicable, since they only provide information on nutrient bioavailability in dredged material, not the significance of discharge, as conventional bioassays are run for long periods compared to the duration of dredged material perturbation. A bioassay is proposed; based on nonlinear diffusion data observed in the field, approximating changing concentration-time relationships at disposal sites. Elutriates were prepared by shaking sediment with site water, then allowing it to settle. After centrifugation and filtration, samples were inoculated with *Dunaliella tertiolecta* or *Selenastrum capricornutum*. At times selected by analysis of dye diffusion data, cultures were diluted to approximate what may happen at the disposal sites. Results with *Dunaliella* suggested that open-water disposal of Mobile Harbor (Alabama) sediments would have no significant effect on algal population; concentrated elutriates were toxic to *Dunaliella*. It is concluded that inhibitory and stimulatory additions would not significantly affect algae when dilution rates at open-water sites are considered. The method could provide information to help determine the appropriate method of dredged material disposal. (Abstract-Buchanan-Davidson-Wisconsin WRSIC).

0098-a

Plumb, R. H., Jr., and G. F. Lee. 1974. Phosphate, algae, and taconite tailings in the western arm of Lake Superior. Proc. 17th Conf. Great Lakes Res. 17:823-836.

phosphate, tailings, taconite, lake waters, algal assay, stimulation, toxicity, eutrophication, bottle test, algae, *Selenastrum capricornutum*.

The purpose of this investigation was to evaluate the significance and availability of phosphate associated with taconite tailings that are presently being discharged into the Minnesota waters of Lake Superior. Laboratory leaching studies showed that phosphate would not be leached from tailings in measurable quantities during a three year period and that phosphate from this source is insignificant compared to other sources in the study area. In addition, laboratory bioassays with *Selenastrum* and Lake Superior algae as well as *in situ* bioassays demonstrated tailings to be non-stimulatory and non-toxic. Since an observation of the primary productivity distribution in the western arm of Lake Superior showed no apparent effect in the discharge area and agreed with the measured and calculated phosphate flux distribution, it was concluded that the discharge of taconite tailings would not contribute to the eutrophication of the lake. (Abstract Only-Author).

0098-b

Polesco-Ionasesco, L. 1974. Biomass produced by green algae *Selenastrum bibraianum* and *Chlorella vulgaris*: 157 cultivated in laboratory and out of doors. Lucr. Gradinii Bot. Bureau. 1974:183-190.

algae, *Selenastrum bibraianum*, *Chlorella vulgaris*, algal assay.

NOTE: No other information is available for the above article. A library search conducted by Library of Congress was not sucessful. Errors in the bibliographic citation made retrieval of the article impossible.

0098-c

Polescu, L. 1974. Some physiological processes in the alga *Selenastrum bibraianum*. Analele Universitatii Bucuresti, Seria Stiintele Naturii. 23:63-68.

algae, *Selenastrum bibraianum*, algal assay.

NOTE: No other information is available for above article. A library search conducted through Library of Congress was not sucessful. Errors in the bibliographic citation made retrieval of the article impossible.

0099

Porcella, D. B. 1969. Continuous flow chemostat assays, p. 7-22. *In* E. J. Middlebrooks, T. E. Maloney, C. F. Powers and L. M. Kaack [eds.], Proc. Eutroph. Biostim. Assessmt. Workshop, 19-21 June 1969, Berkeley, Calif. SERL and NERP sponsored.

continuous flow, algal assay, phosphorus, kinetics, algae, *Selenastrum capricornutum*, phytoplankton, *Chlorella* sp., *Anabaena* sp., wastewater, nitrogen, lake waters.

Because their dynamic properties more nearly approximate ecological conditions than did batch cultures, continuous cultures operating in chemostat mode (constant flow-rate) have been utilized in a variety of studies of algal growth, including assessment of biostimulatory responses. Requirements for steady state continuous flow systems and characteristics of ideal chemostats with illustrations of idealized curves from an experiment concerning relative change in algal cell material and limiting phosphorus concentrations are included. In transient phases of chemostat operation, phosphorus concentrations are less than those calculated theoretically, probably due to "luxury" consumption by algae. Considerations of mass balances and kinetics of algal growth, useful in derivation of continuous cultures theory, need be applied to natural ecosystems to elicit useful information on productivity, nutrient cycling, and successional relationships, based on chemostat experiments described, utilizing both *Selenastrum gracile* and natural algal populations as "seed", are areas requiring further study, including the following problems: multiple limiting factors; use of natural "speed" populations; excessive washout and extinction of cultures in bioassay of oligotrophic waters; development of synthetic media; and profiles of biomass analysis and nutrient concentration during transient growth in order to establish appropriate kinetic parameters. (Abstract-Eichhorn-Wisconsin WRSIC).

0100

Porcella, D. B., P. A. Cowan, and E. J. Middlebrooks. 1973. Biological response to detergent and nondetergent phosphorus in sewage: Part I. Water and Sewage Works 120(11):50-67.

detergents, growth rates, limiting nutrients, phosphorus, wastewater, reservoirs, algal assay, algae, *Anabaena flos-aquae*, *Selenastrum capricornutum*.

Sewage effluent from a suburban community of the City of Logan, Utah, was collected during times when detergents were and were not used to ascertain whether the elimination of phosphate detergents would affect algal growth. Algal bioassays were conducted with *Selenastrum capricornutum* and *Anabaena flos-aquae* using nutrient spikes to determine which nutrients were limiting. Bioassays were also performed using secondary treated, detergent-free sewage samples spiked with detergent to observe whether any increase in biostimulation resulted. The nutrient spikes were NH_4Cl, KH_2PO_4, Fe, and trace elements, and NAAM solution for control.

Chemical analysis for the sewage samples showed that restricted use of detergent resulted in 57 percent decrease in phosphorus content; alum treatment also reduced phosphorus content. However, calculated phosphorus concentrations in the bioassay flasks for different dilutions of the secondary and tertiary effluents of the two sewage samples indicated the P content was high as a result of the high level of P in the reservoir water used for dilution. It is concluded that if the P content of the water is naturally high, the addition of more P will not affect algal growth. The results of the algal bioassays are not included in this part of the report. (Abstract-Little-Battelle WRSIC).

0101

Porcella, D. B., P. A. Cowan, and E. J. Middlebrooks. 1973. Biological response to detergent and nondetergent phosphorus in sewage: Part II. Water and Sewage Works 120(12):36-46.

algal assay, wastewater, toxicity, phosphorus, nutrients, algae, Selenastrum capricornutum, Anabaena flos-aquae, reservoirs, growth rates, trace elements, stimulation.

The responses are presented of Anabaena flos-aquae and Selenastrum capricornutum to detergent and nondetergent phosphorus. Maximum growth and mean growth rates are discussed with maximum growth being based upon the first and last biomass measurements during the growth cycle (18 or 21 days). Mean growth rate represents growth over a 7-day period. Correlation between particulate carbon (PC), optical density (OD) and suspended solids (SS) was performed to establish the relations between the individual parameters. Dilution studies were made to detect toxicity, indicated by unusual growth as a function of dilution. Deionized and reservoir water were used to dilute effluents from secondary and tertiary sewage treatment. In summary, the results indicate the following conclusions: (1) no differences exist between baseline and test samples; (2) response was greater in secondary than tertiary effluents; and (3) iron and trace elements appeared to stimulate slightly greater growth. Samples of various wash products were added to a solution of two percent test secondary effluent in reservoir water. The results showed no significant difference in maximum growth. It can be concluded that the addition of the wash products to treated sewage did not affect the bioassay response. (Abstract-Holoman-Batelle WRSIC).

0102

Porcella, D. B., P. Grau, C. H. Huang, J. Radimsky, D. F. Toerien, and E. A. Pearson. 1970. Provisional algal assay procedures: First annual report. Sanitary Engr. Research Lab, SERL Report, Berkeley, Calif. SERL Report No. 70-8. 180 p.

eutrophication, algal assay, growth kinetics, continuous flow, bottle test, nutrients, growth rates, Michaelis-Menten equation, rivers, lake waters, wastewater, algae, Selenastrum capricornutum.

This report is a detailed coverage of the algal assay procedure with emphasis on a comparison of batch and chemostat assays. The procedures are given for both methods, including actual experiments conducted using the test alga Selenastrum capricornutum and the batch (bottle test) assay and the chemostat assay. An outlined conclusion is given by the authors. (Abstract-Leischman-EPA).

0102-a

Premazzi, G., O. Ravera, and A. Lepers. 1978. A modified turbidostatic system for algal population studies. Mitt. Int. Ver. Limnol. 21:41-49.

algal assay, continuous flow, algae, Selenastrum minutum, growth rates.

A new type of turbidostats described with these important advantages: the population density is controlled by a photodetector sensor with one threshold and the difference between the photoelectrical relays is minimized. The system, in addition to being able to compare the growth rate of algal population cultivated on media with different macro- and/or micronutrients, may be used to record quantitatively and continuously the effects of any pollutant on the primary production of algal populations. The advantage to adopt this turbidostat has been demonstrated by the results obtained from experiment on the effects of low concentrations of Cd salt on a population of Selenastrum minutum. (Summary-Author).

0103

Proctor and Gamble. 1968. Proctor and Gamble laboratory data: PAAP test. Unpublished report.

algal assay, bottle test, wastewater, lake waters, nutrients, algae, Selenastrum capricornutum.

Not available for reproduction as requested by author.

0104

Rawlings, G. D. 1978. Source assessment: Textile plant wastewater toxics study - Phase I. U.S. Environmental Protection Agency, Washington, D. C. Technology Series EPA-600/2-78-004h. 153 p.

wastewater, pollution, toxicity, stimulation, algal assay, bottle test, nutrients, inhibition, algae, *Selenastrum capricornutum*.

The purpose of this study was to provide chemical and toxicological baseline data on wastewater samples collected from textile plants in the United States. Raw waste and secondary effluent wastewater samples were analyzed for 129 consent decree priority pollutants, effluent guidelines criteria pollutants, and nutrients; Level I chemical analyses were also performed. Secondary effluent samples from the 23 plants selected for study in the EPA/ATMI BATEA Study (American Textile Manufacturers Institute/best available technology economically achievable) (Grant No. 804329) were submitted for the following bioassays: mutagenicity, cytotoxicity, clonal assay, freshwater ecology series (fathead minnows, *Daphnia*, and algae), marine ecology series (sheepshead minnows, grass shrimp, and algae), 14-day rat acute toxicity, and soil microcosm. Since this was a screening study, samples of the textile plant intake water were not collected for chemical analysis.

Based on the bioassay results, 10 of the 23 textile plants were found to have secondary effluents sufficiently toxic to proceed to a second phase of the study. In the second phase, samples will be collected from these 10 plants to determine the level of toxicity removal attained by selected tertiary treatment technologies. (Abstract-Author).

0104-a

Reynolds, J. H., E. J. Middlebrooks, and D. B. Porcella. 1973. A continuous flow kinetic model to predict the effects of temperature on the toxicity of oil refinery waste to algae. Utah Water Res. Lab., NTIS, PB-239699, U. S. Dept. Commerce, Springfield, VA., 42 p.

oil, refinery, temperature, algal assay, continuous flow, wastewater, mathematical model, inhibition, toxicity, algae, kinetics, *Selenastrum capricornutum*.

Many industries, such as pulp and paper mills, tanneries, and oil refineries, discharge toxic wastes at extremely high temperatures. The temperature of an oil refinery waste may range from 22°C (71°F) to 41°C (106°F). These high temperatures could have a significant effect on the toxicity of a given waste. It is therefore necessary to develop relationships which will evaluate and predict the effects of increased temperature on the toxicity of various wastes.

Equations have been developed utilizing continuous flow stirred tank reactor (chemostat) kinetics and enzyme inhibition kinetics to describe the effects of temperature on toxicity to microorganisms. These equations were tested by semi-continuous and continuous flow experiments with phenol and the algae *Selenastrum capricornutum*.

The data obtained were fitted to competitive, uncompetitive, and non-competitive inhibitor models. The results indicated that phenol exerts a competitive inhibition effect on the growth of *S. capricornutum*.

Comparison of the competitive inhibition constants, K_I, for *S. capricornutum* exposed to phenol at 20°C, 24°C, and 28°C, indicate that phenol toxicity increases with an increase in temperature according to the Arrhenius function. (Abstract-Author).

0104-b

Reynolds, J. H., E. J. Middlebrooks, D. B. Porcella, and W. J. Grenney. 1974. A continuous flow kinetic model to predict the effects of temperature on the toxicity of waste to algae. Utah Water Res. Lab., NTIS, PB-239194, U.S. Dept. Commerce, Springfield, VA., 112 p.

continuous flow, toxicity, temperature, oil, wastewater, inhibition, phenol, growth rates, algal assay, uptake, kinetics, algae, *Selenastrum capricornutum*.

A continuous flow kinetic model to describe and predict the effects of temperature on the toxicity of a specific oil refinery waste to the green alga, *Selenastrum capricornutum*, is developed. The model is based on enzyme inhibition kinetics and is developed using semi-continuous flow and continuous flow algal cultures grown between 20°C (68°F) and 33°C (91°F). Phenol is employed as the controlling inhibitor or toxicant. The model is applied to continuous flow algal cultures exposed to an actual oil refinery waste.

In addition, the maximum specific growth rates, μ, the half saturation constants, K_s, and the nutrient utilization constants, K_A and K_B, for two luxury uptake functions are determined for the alga, *Selenastrum capricornu-*

tum, growing in an ammonium-nitrogen limited environment between 20°C (68°F) and 33°C (91°F).

Results indicate that phenol and the oil refinery waste studied exert competitive inhibition of *Selenastrum capricornutum*, and that phenol is more toxic at 24°C (75°F) than at either 20°C (68°F) or 28°C (81°F). In addition, the maximum specific growth rate, $\hat{\mu}$, has a maximum value between 24°C (75°F) and 27°C (81°F). Also, the ammonium-nitrogen half saturation constant, K_s, does not vary with temperature between 20°C (68°F) and 33°C (91°F). The variation of the nutrient utilization constants, K_A and K_B, for the luxury uptake functions is similar to the variation of the maximum specific growth rate, $\hat{\mu}$, for the temperature range studied. (Abstract-Author).

0105

Reynolds, J. H., E. J. Middlebrooks, D. B. Porcella, and W. J. Grenney. 1975. Effects of temperature on growth constants of *Selenastrum capricornutum*. J. Water Pollut. Control Fed. 47(10):2420-2436.

algae, kinetics, *Selenastrum capricornutum*, temperature, bottle test, continuous flow, nitrogen, growth rates, uptake, algal assay.

The alga *Selenastrum capricornutum* Printz has been specified for use in bioassays by the Environmental Protection Agency. Continuous-flow (chemostat) experiments conducted at 20°, 24°, 28°, and 33°C indicated that *S. capricornutum* exhibits luxury uptake of ammonium-nitrogen. The luxury uptake of the organism may be described by either a first-order or a hyperbolic function. The nutrient utilization constants for both luxury uptake functions have a maximum value of 3.16 and 2.46 days, respectively, at 24°C. The constants decrease as the temperature is increased or decreased from 24°C. The maximum specific growth rates of the organism at 20°, 24°, 27°, 28°, and 33°C are, respectively, 1.365, 1.992, 1.412, 1.390, and 1.274 days, the maximum occurring between 24° and 27°C. The value of the ammonium-nitrogen half-saturation constant was constant between 20° and 33°C with an average value of 5.356 µg/l NH_4N. (Abstract-JWPCF).

0106

Reynolds, J. H., E. J. Middlebrooks, D. B. Porcella, and W. J. Grenney. 1975. Effects of temperature on oil refinery waste toxicity. J. Water Pollut. Control Fed. 47(11):2674-2693.

algae, inhibition, mathematical model, oil, phenol, refinery, temperature, toxicity, Selenastrum capricornutum, wastewater, continuous flow, algal assay.

A continuous flow kinetic model was developed to describe and predict the effects of temperature on the toxicity of a specific oil refinery waste to the green alga, Selenastrum capricornutum. The model is based on Michaelis-Menten enzyme inhibition kinetics with ammonium nitrogen-limited, continuous flow algal cultures between 20° and 28°C. Phenol is the controlling inhibitor. The model was applied to continuous flow algal cultures exposed to an actual oil refinery waste. The phenol and oil refinery waste studied exerted competitive inhibition of Selenastrum capricornutum. Phenol was more toxic at 24°C than at either 20° or 28°C. The particular oil refinery waste used was approximately 10 times more toxic than pure phenol to Selenastrum capricornutum. (Abstract-JWPCF).

0107

Reynolds, J. H., E. J. Middlebrooks, D. B. Porcella, and W. J. Grenney. 1976. Comparison of semi-continuous flow bioassays, p. 241-266. In E. J. Middlebrooks, D. H. Falkenborg, and T. E. Maloney [eds.], Biostimulation and nutrient assessment, Ann Arbor Sci., Mich.

algal assay, continuous flow, growth rates, mathematical model, temperature, phenol, toxicity, inhibition, algae, Selenastrum capricornutum.

In semi-continuous flow cultures the organism specific growth rate, substrate concentration, and cell concentration vary, while in continuous flow cultures there is a steady state where organism specific growth rate, substrate concentration, and cell concentration are theoretically constant. If the withdrawal period in semi-continuous flow cultures is small enough, continuous flow cultures will be approximated. Actually continuous flow cultures may be considered as a special case of semi-continuous cultures where number of withdrawals per unit time is infinity. The critical factor is the time between withdrawal of culture content and addition of fresh substrates. An experiment was conducted to develop a mathematical model to predict temperature effects on phenol toxicity to Selenastrum capricornutum and to compare semi-continuous and continuous flow cultures. Both methods showed that phenol was more toxic at 24°C than at 20°C or 28°C but the level of toxicity indicated by continuous flow competitive inhibition constants was greater than that indicated by the semi-continuous flow constants. Continuous flow data showed that phenol was more toxic as the hydraulic residence time increased. These differences may be due to variation in the organism specific growth rate which is observed in semi-continuous cultures and may mask certain toxic effects. (Abstract-Buchanan-Davidson-Wisconsin WRSIC).

0108

Rodhe, W. 1978. Algae in culture and nature. Mitt. Int. Ver. Limnol. 21:7-20.

algal assay, cultures, media, limiting nutrients, nutrients, lake waters, PAAP, Z-8, experimental design, algae, _Selenastrum capricornutum_.

The report is a general discussion of the use of algal assays; the development, continuation and future outlook. The author discusses the development of media and the two media used now in algal assays, Z-8 and PAAP; also the test organism, _Selenastrum capricornutum_; nutrients; growth interactions; criteria of the algal cultures; and practical and theoretical conclusions. (Abstract-Leischman-EPA).

0108-a

Ryding, S. O., A. Claesson, and A. Forsberg. 1978. Research on recovery of polluted lakes. II. Biomass budgets using natural and potential (algal assay) chlorophyll _a_. Verh. Int. Ver. Limnol. 20(2):833-839.

lake waters, chlorophyll _a_, algal assay, nitrogen, phosphorus, algae, _Selenastrum capricornutum_, nutrients.

Biomass budgets in terms of total chlorophyll _a_ (= natural + potential chlorophyll _a_), are presented for four polluted Swedish lakes, where nutrient input was reduced due to chemical treatment of wastewater and sewage diversion. A fertilizing capacity-availability-biomass budget (F.A.B.)-diagram was constructed to illustrate the mass balance of the nutrients available for algal growth.

The contributions from the wastewater treatment plant ranged between 11--40 o/o, 11--53 o/o and 9--78 o/o of the total external loading for N, P and total chlorophyll _a_, respectively. For one lake the monthly variation in biomass transports are also illustrated. Comparisons are made between nutrient and biomass budgets. (Summary-Author).

0109

Sachdev, D. R. 1973. Effect of organic fractions from secondary effluent on algal growth. Ph.D. thesis, Rensselaer Polytech. Inst., Troy, N.Y. 402 p.

wastewater, organics, fractionation, stimulation, inhibition, phosphorus, nitrogen, algae, Selenastrum capricornutum, Anabaena flos-aquae, Microcystis aeruginosa, carbon-14, bottle test, algal assay.

An evaluation regarding the effect of sewage effluents and their organic fractions on three test algal species, Selenastrum capricornutum (a green alga), Anabaena flos-aquae (a nitrogen fixing blue-green alga) and Microcystis aeruginosa (a non-nitrogen fixing blue-green alga), was made. Effluents of domestic wastes from water pollution control facilities (a trickling filter plant in Lake George, N.Y., and a contact stabilization plant, Clifton Knolls, in Elnora, N.Y) were selected for study. The sewage effluents were collected, membrane filtered (0.45 μ) and then concentrated by the freeze-drying technique in order to get a sufficient amount of organics in the concentrated effluent.

Sephadex Gels G-10, G-15, and G-25 were employed for separation. Fractionation of concentrated effluent in G-10 resulted in five well defined organic fractions. The frontal peak of G-10 was chromatographed on G-15 yielding two organic fractions. The frontal peak of G-15 was fractionated on G-25 and only one organic fraction was obtained. The apparent molecular weight (AMW) was assigned to each fraction from the calibration curves developed for each Sephadex Gel Column. Soluble organics, in the concentrated effluents, with AMW <700 formed 62.2% (Lake George) and 58.5% (Clifton Knolls) and of AMW >5000 were 20.4% (Lake George) and 28.8% (Clifton Knolls).

Algal cultures were grown in New Algal Assay Medium under controlled environments of temperature, light and shaking. Concentrated effluents and their organic fractions were added to the cultures of the three algal test species and their effect was followed by daily absorbance and ^{14}C (cpm) measurements. ^{14}C experiments were conducted in two different ways.

Maximum specific growth rate (μ_{max}) and maximum standing crop were employed to evaluate the growth response of the test algal species. μ_{max} was calculated by EPA (1971) method and regression analysis. Maximum standing crop was assumed to have been achieved in a flask when daily increase in biomass was less than 5%. Results were considered significant if deviation from the control was more than ±10%.

Based upon the results generated, it was concluded that in general both concentrated effluents and organic fractions with AMW < 700 indicated significant stimulatory effect to μ_{max} and maximum standing crop. Organic fractions of AMW <700 had varying effects on different algal species, some showed stimulation, some depicted inhibition while others had no effect.

The stimulatory effect was due to the organic compounds in the fractions. These organic compounds acted as growth substances and substrates (nutrients). In some organic fractions they acted in both capacities.

It was also noted that removal of organics from concentrated effluents or reduction of their concentration by dilution could arrest algal growth.

The dual nature of organic fractions was noticed. Some organic fractions would act differently when alone than when in combination with others. Some organic fractions were stimulatory to one alga and inhibited another.

Some of the organic fractions showed better stimulation than the concentrated effluent.

The addition of phosphorus and nitrogen did not significantly affect the algal growth response and it was concluded that the effect, noted in this work was due to organic compounds in the fractions. (Abstract-Dissertation Abstracts Internatl.).

0110

Sachdev, D. R., and N. L. Clesceri. 1978. Effects of organic fractions from secondary effluent on _Selenastrum_ _capricornutum_ (Kutz). J. Water Pollut. Control Fed. 50(7):1810-1820.

wastewater, fractionation, organics, stimulation, algal assay, nitrogen, phosphorus, inorganic carbon, inhibition, algae, _Selenastrum_ _capricornutum_.

The secondary treated effluents from two domestic wastewater treatment facilities were membrane filtered (0.45μ), concentrated via freeze-drying technique, and then fractionated. Sephadex gels G-10, G-15, and G-25 were employed for fractionation. Effect of the concentrated effluents and their various organic fractions on the maximum specific growth rate (μ_{max}) and maximum standing crop of _Selenastrum_ _capricornutum_ was investigated. In general the concentrated effluents and the organic fractions with apparent molecular weight >700 indicated stimulatory effect on μ_{max} and the maximum standing crop. The stimulation of S. capricornutum was the result of the organic compounds in these fractions. The supplemental nitrogen, phosphorus or inorganic carbon added to the medium from the addition of the organic fractions did not contribute to the stimulation of this alga. Neither the concentrated effluents nor any of the organic fraction showed any inhibitory effect on S. capricornutum. (Abstract-JWPCF).

0111

Saldick, J., and J. F. Jadlocki. 1978. Solubilization of biological available phosphorus by autoclaving Selenastrum. Mitt. Int. Ver. Limnol. 21:50-55.

phosphorus, algal assay, bottle test, nutrients, pretreatment, inhibition, algae, Selenastrum capricornutum, solubilization.

Autoclaving solubilized 70-85% of the phosphorus contained in Selenastrum but only 15-45% appeared as soluble reactive phosphorus (SRP). The soluble but non-reactive phosphate (TSP minus SRP) was generally not available for cell replication in 21 days in the Algal Assay Procedure Bottle Test (US EPA, 1971), but there were some exceptions in which a portion of this fraction was used. SRP was consistently available in the 21-day bottle test. Retardation of growth rate due to removal of essential micronutrient(s) other than iron was observed when the cell population at the time of autoclaving was sufficiently high. Recently acquired surplus phosphorus was released as SRP in greater yield (~50%) by autoclaving than older surplus phosphorus.

The nutritional state of the algae present in bottle test water samples influences the test results and must be considered in their interpretation. (Summary-Author).

0112

Schelske, C. L., E. D. Rothman, and M. S. Simmons. 1978. Comparison of bioassay procedures for growth-limiting nutrients in the Laurentian Great Lakes. Mitt. Int. Ver. Limnol. 21:65-80.

phosphorus, algal assay, nitrogen, lake waters, algae, Selenastrum capricornutum Printz, phytoplankton, bottle test.

A series of experiments conducted on the effects of phosphorus, nitrogen and silica on the growth of algae showed that phytoplankton were phosphorus limited in Lake Huron and Lake Michigan but not in Lake Erie. Three types of experiments were used: a spiked test with Selenastrum capricornutum Printz, a spiked test with natural phytoplankton assemblages and a fixed-level test with natural phytoplankton assemblages in which depleted nutrients were replenished periodically to maintain specified levels. A replicated factorial design was used in all experiments. Levels of nutrients were ambient (existing levels), plus three additions of phosphorus ranging from 5 to 15 μg/liter, two additions of silica, 0.8 and 1.6 mg SiO_2/liter, and one addition of nitrate, 0.30 mg N/liter. Although other variables were used, changes in chlorophyll standing crops were utilized most frequently to determine whether treatments affected algal growth. Results indicate that experiments with natural populations can be conducted either in the field or laboratory and that the naturally occurring phytoplankton assemblages respond to treatments containing low levels of nutrients and are not excluded by experimental conditions. Such experiments with small nutrient additions therefore can be used to obtain valuable information on the ecology of specific populations in natural phytoplankton assemblages. (Summary-Author).

0113

Scherfig, J., P. S. Dixon, R. Appleman, and C. A. Justice. 1973. Effect of phosphorus removal processes on algal growth. U.S. Environmental Protection Agency, Washington, D. C. EPA-660/3-73-015. 81 p.

algal assay, phosphorus, bottle test, continuous flow, wastewater, eutrophication, limiting nutrients, algae, Selenastrum capricornutum, phytoplankton.

Laboratory studies were conducted to improve algal assay techniques for use in evaluation of sewage treatment processes.

Laboratory studies (batch and continuous cultures) were conducted at the Santee California water reclamation plant to evaluate the effect of tertiary waste treatment processes on the amount of algal growth in the treated effluent.

Laboratory studies were also conducted to determine the growth limiting nutrients in each type of tertiary effluent.

Field tests were conducted using special study ponds and the results of the field tests were compared with the laboratory test results. The laboratory and field tests showed the same relative ranking for the treated effluents. (Abstract-Author).

0113-a

Scott, W. E., M. T. Seaman, A. D. Connell, S. I. Kohlmeyer, and D. F. Toerien. 1977. The limnology of some South African impoundments. I. The physico-chemical limnology of Hartbeespoort Dam. J. Limnol. Soc. South Afr. 3(2):43-58.

eutrophication, lake waters, impoundment, nutrients, nitrogen, phosphate, algal assay, algae, Selenastrum capricornutum Printz, bottle test.

Hartbeespoort Dam is a eutrophic, warm monomictic lake with overturn occurring in April. The lake is stratified for about six months of the year and in late summer the stratification appears stable. Light penetration in the dam depends both on the inflow of turbid water as well as on the magnitude of the algal population. In summer the surface waters are often supersaturated with oxygen, while the hypolimnion is anaerobic. The concentrations of nitrogen compounds, phosphate, Na, K, Ca, Cl and SO_4 are higher in waters entering the dam via the Crocodile River. Nitrogen compounds are the primary growth-limiting nutrients for algal growth and phosphate is the secondary growth-limiting nutrient. Phosphate concentrations in the water have increased approximately a hundredfold since 1928 and NO_3-N concentrations about threefold. The pH as well as the mineral content of the dam have also increased over the last 50 years. (Summary-Author).

0114

Sherrard, J. H., and J. L. Hoyle. 1977. A productivity study of the Roanoke River above Niagara Dam in Virginia. Va. Water Res. Res. Center, Blacksburg. Bulletin No. 104, 65 p.

rivers, lake waters, eutrophication, algal assay, bottle test, nutrients, nitrogen, phosphorus, limiting nutrients, growth rates, wastewater, algae, Selenastrum capricornutum, Anabaena flos-aquae, water quality.

Deterioration of the water quality in the upper end of the Roanoke River of Smith Mountain Lake due to eutrophication has caused much concern. The Smith Mountain Lake impoundment was constructed in 1965 as a pump storage facility to generate electrical energy and has served as a major recreational area for Southwestern Virginia.

Eight sampling stations were established along the length of the Roanoke River from an agricultural area in Montgomery County through the urban area of Roanoke-Salem to Niagara Dam above Smith Mountain Lake. On 12 different occasions, approximately once a month, grab samples were taken at the sampling stations. Samples were analyzed for general chemical parameters and for nitrogen and phosphorus. The Algal Assay Procedure Bottle Test [Environmental Protection Agency, 1971] was conducted with the samples, and in addition, with samples in which phosphorus and/or nitrogen spikes were added to determine potential productivity and to determine which element was limiting productivity.

Results of this investigation demonstrate that the algal assay bottle test is useful in correlating phosphorus concentrations with algal growth rate. Phosphorus was found to limit the algal growth in the Roanoke River above the Roanoke wastewater treatment plant for _Selenastrum_ _capricornutum_ and _Anabaena_ _flos-aquae_, the two algal species used in this study. Maximum specific growth rates tended to increase with increasing phosphorus concentration. (Abstract-Author).

0115

Shiroyama, T., W. E. Miller, and J. C. Greene. 1975. Effect of nitrogen and phosphorus on the growth of _Selenastrum_ _capricornutum_ Printz, p. 132-142. _In_ Proc. Biostim. Nutr. Assessmt. Workshop, 16-17 October 1973, U.S. Environmental Protection Agency, Corvallis, Oregon. EPA-660/3-75-034.

algal assay, bottle test, nutrients, nitrogen, phosphorus, limiting nutrients, stimulation, eutrophication, algae, _Selenastrum_ _capricornutum_ Printz.

The report discusses the use of the algal assay bottle test to determine effects of nitrogen and phosphorus on algal growth. The test species used was _Selenastrum_ _capricornutum_. Tests were conducted in a defined medium. Nitrogen and phosphorus were added as nutrient spikes. The authors found that: in the analysis of growth rates, maximum uptake of the nutrients nitrogen and phosphorus occurred in the initial 5 days of growth; the increased growth rates of the alga, with phosphorus additions indicated assimilation of phosphorus more rapidly than nitrogen; there was a defined linear relationship between produced biomass and concentrations of the nutrients; when all essential nutrients were present, toxicants were absent, and nitrogen and phosphorus requirements were determined, maximum growth could be predicted within ±20% of the actual yield. (Abstract-Leischman-EPA).

0116

Shiroyama, T., W. E. Miller, and J. C. Greene. 1976. Comparison of the algal growth responses of *Selenastrum capricornutum* Printz and *Anabaena flos-aquae* (Lyngb.) De Brebisson in waters collected from Shagawa Lake, Minnesota, p. 127-148. *In* E. J. Middlebrooks, D. H. Falkenborg, and T. E. Maloney [eds.], Biostimulation and nutrient assessment, Ann Arbor Sci., Mich.

algal assay, bottle test, growth rates, nutrients, nitrogen, phosphorus, wastewater, limiting nutrients, algae, *Anabaena flos-aquae* (Lyngb.) De Brebisson, *Selenastrum capricornutum* Printz, lake waters.

The growth response of *Anabaena flos-aquae* was studied to determine its reliability as a test alga and to compare its growth response to that of *Selenastrum capricornutum* in water samples from Shagawa Lake, Minnesota. Results showed that *A. flos-aquae* can be used as a test organism to define growth potential and nutrient status of lake waters. For counting, sonication was more effecient than blending to fragment *A. flos-aquae*, but it damaged cells so cannot be used to prepare an inoculum. Water containing more than 0.010 mg/l ortho-phosphate yielded 0.45 mg/l dry weight *A. flos-aquae* per 0.001 mg/l phosphorus. Filtered and autoclaved-filtered water samples were primarily nitrogen-limited for *S. capricornutum* support. The *A. flos-aquae* growth response indicated constituents other than nitrogen and phosphorus limited its growth in filtered samples; addition of phosphorus stimulated growth to the maximum predicted values in autoclaved-filtered samples. Comparative analysis of algal growth responses suggested that more than one test species should be used to determine the growth potential or nutritional status of a test water. If the presence of a biologically produced toxin is suspected, filtered and autoclaved-filtered samples must be analyzed to determine the presence of a biologically produced growth-inhibitor. (Abstract-Buchanan-Davidson-Wisconsin WRSIC).

0117

Shoaf, W. T., and B. W. Lium. 1976. An evaluation of methods for measuring algal growth. J. Res. U.S. Geological Survey 4(4):497-504.

algal assay, growth rates, electronic particle counter, fluorescence, algae, *Chlorella vulgaris*, *Selenastrum capricornutum*, *Chlamydomonas reinhardtii*, *Scenedesmus quadricauda*, *Anacystis nidulans*.

Algal growth was expressed as milligrams dry weight per litre with use of the electronic particle counter for determining cell number and cell volume. A gravimetric constant in milligrams dry weight per cubic micrometer was determined. This constant was applicable throughout the growth curve since there was a linear relationship between total cell weight and total cell volume. Cell volume must be considered in measuring growth because there is a change in average cell volume in addition to an increase in cell numbers throughout the growth curve. *Chlorella vulgaris* and *Selenastrum capricornutum* exhibited similar growth curves when growth was expressed as cells per litre or as milligrams dry weight per litre. *Chlamydomonas reinhardtii* appeared to have a lower algal growth potential when measured in cells per litre but had the same growth potential as *C. vulgaris* and *S. capricornutum* when expressed as milligrams dry weight per litre. The growth rate for *Scenedesmus quadricauda* was less than the other three, regardless of how the results were expressed. The image analysis system was used to count different types of algae in a mixed culture. *C. reinhardtii*, *C. vulgaris* and *Anacystis nidulans* were not grossly distorted by the sample preparation method and could be distinguished from one another by variations in morphology. The counts could be separated by computing the ratio of area to length for each particle and by allowing the computer to sort and count each of the three types. Typical ratios ranged from 5 to 7 for *C. reinhardtii*, 2 to 4 *C. vulgaris*, and 0.2 to 1.3 for *A. nidulans*. The number of types to be counted in a mixture was limited only by the ability to apply shape factors without having an overlap between each type. Both the image analysis and electronic particle counter produced equal count and size measurements. Fluorescence was a sensitive measure of growth on a laboratory prepared medium since there was a logarithmic relationship between fluorescence and cells per millilitre for both *C. vulgaris* and *S. capricornutum*. Fluorescence measurements were not satisfactory for determining growth in samples of varying chemical composition (which occurs in natural samples) because the chlorophyll-to-cell ratio varies with media composition. This was especially pronounced with *C. vulgaris*. (Abstract-Author).

0118

Shoaf, W. T., and B. W. Lium. 1976. Improved extraction of chlorophyll *a* and *b* from algae using dimethyl sulfoxide. Limnol. Oceanogr. 21(6):926-928.

chlorophyll *a*, chlorophyll *b*, DMSO, dimethyl sulfoxide, algae, *Selenastrum capricornutum*, *Chlorella pyrenoidosa*, *Tetraedron bitridens*, *Scenedesmus quadricauda*, *Oocystis marssonii*, *Ankistrodesmus braunii*, *Chlamydomonas reinhardtii*, *Anacytis nidulans*, *Anabaena flos-aquae*, *Fremyella diplosiphon*, *Cyclotella* sp., *Nitzschia* sp., algal assay, diatoms.

Dimethyl sulfoxide (DMSO) and 90% acetone extracted equal amounts of chlorophyll from diatoms and blue-green algae, but DMSO was superior to 90% acetone for all green algae tested giving 2-60 times more chlorophyll depending on the species. The absorbance spectra of pure chlorophyll *a* (and *b*) from 600 nm to 750 nm were identical whether dissolved in 90% acetone or a mixture of DMSO and 90% acetone (1:1 v/v). Thus, several equations for estimating chlorophyll concentration based on extinction in 90% acetone are applicable with this solvent. (Abstract-Author).

0119

Skulberg, O. M. 1964. Algal problems related to the eutrophication of European water supplies, and a bioassay method to assess fertilizing influences of pollution on inland waters, p. 262-299. *In* D. F. Jackson [ed.], Algae and man, Plenum Press, New York.

algal assay, eutrophication, pollution, wastewater, lake waters, rivers, phytoplankton, algae, *Selenastrum capricornutum*, *Ankistrodesmus* sp.

The author lists among the problems caused by excessive algal growth, filter-blocking, impartation of taste and odor to water supplies, discoloration of water, provision of nutrients for undesirable heterotrophs in pipes, and formation of sediments in lakes. To illustrate differences in trophic status among lakes, he compares two lakes in Norway:Lake Maridalsvannet (3.9 square kilometers/maximum depth, 45 meters) which provides Oslo with drinking water, is oligotrophic, and shows little annual variation in its low concentrations of dissolved nutrients. Lake Borrevannet (1.8 square km/max depth, 16 meters) is eutrophic and shows important annual changes with respect to chemistry and water quality. Author states that Lake Lugano, Northern Italy, has eutrophied due to pollution enrichment, citing classic example of Lake Zurich as evidence that lacustrine trophic changes are best recorded in the sediments. Utilizing Selenastrum capricornutum, (or Ankistrodesmus) an organism requiring only a few days to attain peak growth, author devised a bioassay to assess effects and concentrations of nutrients in natural waters. Such an approach would appear to have its greatest value in comparative limnological studies. (Abstract-Eichhorn-Wisconsin WRSIC).

0119-a

Skulberg, O. M. 1966. Algal cultures as a means to assess the fertilizing influence of pollution, p. 113-138. In O. Jaag and H. Liebmann [co-chm], Advances in water pollution research, Water Poll. Control Fed., Washington, D. C.

pollution, wastewater, rivers, lake waters, fjord, algal assay, nutrients, cultures, media, algae, Selenastrum capricornutum, Skeletonema costatum, Oscillatoria Vaucher, Chlorella ovalis Butcher.

The paper is a discussion of experiments, and the results, conducted to study eutrophication in relation to the pollution of Norwegian inland waters and fjords. Algal assays were used to determine the nutrients available to support algal productivity. The presentation includes discussion of the test organisms, Selenastrum capricornutum, Skeletonema costatum, Oscillatoria sp., and Chlorella ovalis Butcher; the culture media, Z-8; growth experiments in the media and inland waters; and, the possibilities and limitations in using algal assays. (Abstract-Leischman-EPA).

0120

Skulberg, O. M. 1973. A comparative investigation of water from 15 European lakes, p. 85-100. In Algal assays in water pollution research, Proc. Nordic Symp., 25-26 October 1972, Oslo, Norway. NORDFORSK, Secretariat of Environmental Sciences.

algal assay, lake waters, eutrophication, water quality, COD, phosphorus, nitrogen, nutrients, classification, algae, Selenastrum capricornutum.

A report on investigations of the status of 15 European lakes. The algal assay was used to assess the effects of enrichment of the lake waters as a result of human influence. Methods of sampling; preparation of samples; analyses of nutrients; and, growth results for the test alga Selenastrum capricornutum are given. A classification of the lakes in regards to the trophic status was possible from the results obtained. (Abstract-Leischman-EPA).

0121

Skulberg, O. M. 1974. Observation and monitoring of water quality by use of experimental biological methods. Verh. Int. Ver. Limnol. 19(3):2053-2063.

algal assay, pollution, wastewater, eutrophication, nutrients, water quality, algae, Selenastrum capricornutum.

The report is a discussion of the importance and the implications for using experimental biological methods to assess the effects of pollution on water systems. The author emphasizes use of observations of biological populations coupled with physical and chemical characteristics of the waters, as a good practice in water quality management. Algal assays as a comparative limnological method are discussed. Data and results from past studies conducted on 15 European lakes are presented. (Abstract-Leischman-EPA).

0122

Skulberg, O. M. 1976. Culture collection of algae. Norwegian Inst. Water Res. (NIWR) Publ. Q91, Blindern, Norway, 9 p.

cultures, algae, Cyanophyceae, Chlorophyceae, Bacillariophyceae, lake waters, rivers, coastal waters, nutrients, trace elements, algal assay, Selenastrum capricornutum Printz CHL 1, Selenastrum westii G. M. Smith CHL 17.

The report is a brief procedure of the collection of 57 isolate algal species, most originating from Norwegian waters. A description of the media used and the growth conditions are given. Included is a list of all the species collected, isolated, the location and year of collection, and the media used for each species. (Abstract-Leischman-EPA).

0123

Smock, L. A., D. L. Stoneburner, and J. R. Clark. 1976. The toxic effects of trinitrotoluene (TNT) and its primary degradation products on two species of algae and the fathead minnow. Water Res. 10(6):537-543.

TNT, trinitrotoluene, algal assay, toxicity, bottle test, algae, *Selenastrum capricornutum*, *Microcystis aeruginosa*, inhibition.

The effects of alpha trinitrotoluene (alpha TNT) and its primary degradation product (TNT cc) commonly referred to as "pink water", were determined on members of two trophic levels. The growth responses of the algae *Selenastrum capricornutum* and *Microcystis aeruginosa* were examined through static bioassays. Death and behavioral responses of the fathead minnow (*Pimephales promelas*) were determined using a proportional diluter. Alpha TNT and TNT cc were both more toxic to the fathead minnow than to either species of alga. Five and 15 mg l^{-1}, inhibited *S. capricornutum* and *M. aeruginosa* growth, respectively. TNT cc inhibited *S. capricornutum* growth at concentrations above 9 mg l^{-1}; it was lethal to *M. aeruginosa* at 50 mg l^{-1}, but stimulated growth at lower concentrations. The 96-h LC_{50} values based on the death response of the fathead minnow to alpha TNT and TNT cc were 2.58 and 1.60 mg l^{-1}, respectively. The 96-h EC_{50} values based on the behavioral responses were 0.46 and 0.64 mg l^{-1}, respectively. There was no response to concentrations of 0.05 mg l^{-1} alpha TNT and 0.07 mg l^{-1} TNT cc. (Abstract-Author).

0124

Smolen, M. D. 1975. Prediction of algal growth potential from chemical nutrient analysis. Va. J. Sci. 26(2):78.

reservoirs, watershed, nutrients, nitrogen, phosphorus, AGP, nitrate, phosphate, ammonia, algal assay, algae, *Selenastrum*, *Anabaena*.

Weekly water samples were obtained from the reservoir and major tributary streams of the Occoquan watershed and analyzed for forms of nitrogen and phosphorus. A portion of these samples were also subjected to AGP analysis using *Anabaena* and *Selenastrum*. Distinctly different responses were noted for the two test species on water samples from several locations. Correlation analysis for AGP yield with chemical nutrient parameters indicates that nitrate and phosphate concentrations relate closely to the AGP yields observed. Although ammonium ion concentration was observed to vary from extreme low to extreme high levels, no significant positive correlation with AGP yield was noted.

A series of curves is presented which show observed AGP yield as a function of nitrate concentration at various phosphate levels. The results indicate that nitrate concentration determines Selenastrum yield when phosphate concentration is greater than 0.010 mg P/L. Anabaena, however, responds to nitrate concentration only when total phosphate concentration is greater than 0.150 mg P/L. (Abstract Only-Author).

0125

Soltero, R. A., A. F. Gasperino, P. H. Williams, and S. R. Thomas. 1975. Response of the Spokane River periphyton community to primary sewage effluent and continued investigation of Long Lake. D.O.E. Project WF-6-75-081. Completion Report. 117 p.

wastewater, rivers, nutrients, reservoirs, nitrogen, phosphorus, eutrophication, temperature, stratification, fractionation, organics, sediments, algal assay, bottle test, algae, Selenastrum capricornutum, phytoplankton.

In part, the study examined the effects of primary sewage effluent from Spokane, Washington on the diatom community in the Spokane River. Both natural and artificial substrates were used at each of those sampling stations, one above and two below the outfall. In terms of individual species abundance, mean diversity indices, floristic similarities between sampling stations and mean autotrophic indices, the diatom community at the last downstream station had begun to recover from the discharge.

Continued investigation of Long Lake and its tributaries has shown that a major source of nutrients influent to the reservoir was the City of Spokane's sewage effluent. Existing mean concentrations of inorganic nitrogen and orthophosphate increased approximately twofold and sevenfold, respectively, below the effluent. Of the total influent phosphate, 22 percent was retained in the reservoir.

The reservoir was again determined to be highly eutrophic. Orthophosphate, nitrate nitrogen, silica and temperature were found to be important variables regulating the size of the phytoplankton community. Long Lake's seasonal phytoplankton periodicity, however, depended primarily upon stratification, turbulence, light penetration and retention time.

Close interval fractionation of Long Lake sediment cores has provided a reliable static record of the reservoir's trophic status since 1958. Organic matter appeared to be stabilized prior to incorporation into the sediments.

Nitrogen and phosphorus concentrations increased 1.4 times whereas diatom production has increased sevenfold. A sediment accumulation rate of 24 mm yr^{-1} was determined and does not appear to be excessive when compared to other reservoirs. Vertical migration, leaching and internal recycling of phosphorus from the sediments should have little effect, if any, on the reservoir's trophic status once secondary sewage treatment with phosphorus removal is initiated by the City of Spokane. (Abstract-Author).

0126

Soltero, R. A., D. M. Kruger, A. F. Gasperino, J. P. Griffin, S. R. Thomas, and P. H. Williams. 1976. Continual investigation of eutrophication in Long Lake, Washington. Verification data for the Long Lake Model. D.O.E. Project WF-6-75-081. Completion Report. 64 p.

lake waters, eutrophication, reservoirs, wastewater, algal assay, bottle test, nutrients, phosphorus, nitrogen, limiting nutrients, heavy metals, inhibition, water quality, phytoplankton, algae, Selenastrum capricornutum Printz.

Long Lake, an impoundment of the Spokane River, was again determined to be eutrophic. The major source of nutrients influent to the reservoir was from the Spokane primary sewage treatment plant. During the study, Long Lake had a mean exchange rate of 27 days, shortest of the four years studied (Soltero, Gasperino and Graham, 1973, 1974; Soltero et al., 1975). Hypolimnetic anoxia was not as extensive as previously determined (Soltero et al., 1973; 1974; 1975) due to lower reservoir retention times and the establishment of an underflow of the Spokane River at stations 1 and 2 during August and September.

Phytoplankton and zooplankton standing crops also approximated those of previous years (Soltero et al., 1973; 1974; 1975) in species diversity, occurrence and abundance. Primary productivity was the lowest of the four years studied (Soltero et al., 1973; 1974; 1975). Reservoir algal assay data indicated nitrogen to be the primary limiting nutrient (57 percent cf the time) and phosphorus the secondary limiting nutrient (30 percent of the time). Nitrogen and phosphorus co-limitation occurred in 13 percent of the samples. Heavy metal inhibition to Selenastrum capricornutum Printz existed in every sample.

Prior studies (Soltero et al., 1973; 1974; 1975) have given a comprehensive portrayal of Long Lake's dynamics and this base-line information has been used by Battelle, Pacific Northwest Laboratories, in the calibration of a water quality model. However, the simulated results must be verified with "actual" field data in order to determine the sensitivity of the model's predictions. The data collected during this study are representative of past years and can be used for the verification of the Battelle water quality model of Long Lake. (Abstract-Author).

0126-a

Sridharan, N., and G. F. Lee. 1974. Algal nutrient limitation in Lake Ontario and tributary waters. Water Res. 11(10):849-858.

nutrients, lake waters, nitrogen, phosphorus, stimulation, rivers, trace elements, limiting nutrients, wastewater, toxicity, eutrophication, algal assay, algae, Selenastrum capricornutum.

In nutrient-spiking studies of Lake Ontario and its tributary rivers of Niagara, Black, Genesee, and Oswego waters both nitrogen and phosphorus were required for stimulation of cultured and natural algae growth. Samples of the Genesee and Oswego Rivers generally showed nitrogen stimulation. Also, many of the samples from these rivers demonstrated stimulation due to the addition of micronutrients. However, care must be exercised in interpreting micronutrient stimulation as the micronutrient solution contained EDTA, a strong complexing agent, which can eliminate toxic elements in the water. Essentially all lake water samples showed phosphorus limitation. It is possible that phosphorus removal at domestic wastewater treatment plants located in these areas could be sufficient to cause phosphorus to be limiting. It is also possible that advanced waste treatment could reduce the concentration of the apparent toxicants in these rivers which would stimulate algae growth in the rivers and nearshore lake waters due to excess of phosphorus present. Even though the potential impact of phosphorus removal cannot be predicted for these rivers and nearshore Lake Ontario waters, it is clear that such a practice will be of some benefit in reducing and possibly reversing the excessive eutrophication trend. (Abstract-Auen-Wisconsin WRSIC).

0126-b

Sridharan, N., and G. F. Lee. 1977. Algal nutrient availability and limitation in Lake Ontario during IFYGL, Part III. Algal nutrient limitation in Lake Ontario during IFYGL. U.S. Environmental Protection Agency, Duluth, Minn., Ecol. Res. Series. EPA-600/3-77-046a. 46 p.

nitrogen, phosphorus, limiting nutrients, phytoplankton, lake waters, rivers, algal assay, bottle test, algae, *Selenastrum capricornutum*.

This study was conducted on the potential significance of nitrogen, phosphorus and micronutrients in limiting planktonic algal growth in Lake Ontario and its major tributaries. Standard algal assay procedures were used. Samples of the open waters of Lake Ontario and Niagara River waters collected during the spring showed phosphorus limitation. By late summer these waters showed both nitrogen and phosphorus limitation. Genesee and Oswego Rivers showed, in general, nitrogen limitation. Samples of the Black River waters showed both nitrogen and phosphorus limitation. (Abstract-Author).

0126-c

Starr, R. C. 1978. The culture collection of algae at the University of Texas at Austin. J. Phycol. Suppl. 14:47-100.

algae, media, cultures, *Selenastrum bibraianum* Reinsch 324 (UTEX), *Selenastrum capricornutum* Printz 1648 (UTEX), *Selenastrum gracile* Reinsch B 325 (UTEX), *Selenastrum minutum* (Näg.) Collins 326 (UTEX), *Selenastrum* 235 (UTEX), *Selenastrum* 747.

An updated, complete list of algae (a variety of strains and their classifications) available to researchers. Sections on maintenance media and special growth media included.

0126-d

Steeman Nielson, E. 1978. Principal aspects concerning the batch technique in algal assays. Mitt. Int. Ver. Limnol. 21:81-87.

algal assay, growth rates, growth kinetics, algae, diatoms, Steeman Nielson and v. Brand technique, *Selenastrum capricornutum*, *Nitzschia palea*.

The report is of experiments conducted using a special batch technique (Steeman Nielson and v. Brand counting technique) to investigate influences of low nutrient concentrations on dilute algal suspensions as might be found in nature. The author felt that the measurement of maximum growth rates in batch cultures were not always the same as real growth rates. The experiments were designed to run during the growth phase where growth rates were constant. The two algae used in the experiments were Selenastrum capricornutum and Nitzschia palea. The test containers were grown under continuous light and alternating light and dark periods. The author found there was little difference in the growth rates of the algae under the continuous light or 12 hr light and 12 hr dark periods (radiance given as 28 quanta x 10^{15} x cm^{-2} x sec^{-1}). Growth curves are given. (Abstract-Leischman-EPA).

0127

Steensland, H. 1973. Application of the dialysis technique in testing possible growth retarding effects on algae in purified wastewater, p. 47-49. In Algal assays in water pollution research, Proc. Nordic Symp., 25-26 October 1972, Oslo, Norway. NORDFORSK, Secretariat of Environmental Sciences.

dialysis, growth rates, wastewater, toxicity, pollution, water quality, Z-8, limiting nutrients, continuous flow, algae, Scenedesmus quadricauda, Selenastrum capricornutum, algal assay.

A brief preliminary report on the use of the dialysis technique to test effects of wastewaters on algal growth. An outlined procedure is presented for the design of the dialysis bags and culture baths. References given. (Abstract-Leischman-EPA).

0128

Steyn, D. J., D. F. Toerien, and J. H. Visser. 1974. Continuous culture algal bioassays. South Afr. J. Sci. 70(9):277-278.

eutrophication, wastewater, limiting nutrients, continuous flow, nitrogen, algal assay, algae, Selenastrum capricornutum.

A brief report of studies conducted using continuous flow algal assays to determine the limiting nutrients of four South African impoundments. Nitrogen was found to be the limiting nutrient in all four impoundments. Results and discussion given. (Abstract-Leischman-EPA).

0128-a

Steyn, D. J., W. E. Scott, D. F. Toerien, and J. H. Visser. 1975. The eutrophication levels of some South African impoundments. I. Rietvlei Dam. Water S.A. 1(2):45-52.

algal assay, impoundment, eutrophication, wastewater, nitrogen, phosphorus, nutrients, bottle test, algae, Selenastrum capricornutum, Anabaena flos-aquae.

Algal bioassay results show Rietvlei Dam to be in a highly eutrophied state. The growth of non-nitrogen-fixing algae is limited by nitrogen, and that of nitrogen-fixing algae by phosphorus. It is predicted that increased discharge of sewage works effluent will increase the eutrophication status of the dam, but an inflow with lower nutrient concentrations will reduce it. The results suggest that the control of phosphorus rather than nitrogen inputs must be considered for eutrophication control, but a more complete understanding of the chemical, physical and biological characteristics of the impoundment is required before a decision can be made as to the best methods of eutrophication control. (Abstract-Author).

0128-b

Steyn, D. J., D. F. Toerien, and J. H. Visser. 1975. Eutrophication levels of some South African impoundments. II. Hartbeespoort Dam. Water S.A. 1(3):93-101.

algal assay, bottle test, AGP, nitrogen, phosphorus, nutrients, eutrophication, impoundment, algae, Selenastrum capricornutum Printz.

Algal bioassay indicated that Hartbeespoort Dam is highly eutrophied because algal growth potentials (AGP) of more than 100 mg l^{-1} and batch culture algal growth rates of 2.1 d^{-1} were recorded. Nitrogen was the primary and phosphorus the secondary growth-limiting nutrient. Addition of secondary sewage effluents linearly increased the AGP at rates of between 7.7 and 13.0 mg l^{-1} percent (v/v) effluents added. These increases could be attributed to an increase in the nitrogen content of the water by the effluent. Addition of sewage effluents did not change the identity of nitrogen as the primary growth-limiting nutrient. Dilution with water of a low nutrient content linearly decreased the AGP. Although nitrogen at present determines the magnitude of algal blooms in the dam, phosphorus control is advocated for the control of eutrophication problems in the dam. (Abstract-Author).

0128-c

Steyn, D. J., D. F. Toerien, and J. H. Visser. 1976. Eutrophication levels of some South African impoundments. III. Roodeplaat Dam. Water S.A. 2(2):1-6.

algal assay, AGP, limiting nutrients, phosphorus, wastewater, nitrogen, algae, Selenastrum capricornutum, impoundment.

Algal bioassays indicated that the waters of Roodeplaat Dam are severely eutrophied since algal growth potentials (AGP) of up to 100 mg/l and batch culture algal growth rates of up to 2.2 d^{-1} were registered with Selenastrum capricornutum as test alga. Phosphorus was usually the primary growth-limiting nutrient in the samples except at the end of winter when nitrogen was primary growth limiting. The secondary limiting nutrient was either nitrogen or phosphorus, depending on the identity of the primary growth-limiting nutrient. Addition of secondary effluent linearly increased the AGP of Roodeplaat Dam water by 7.2 to 12.4 mg/l for each one per cent (v/v) of secondary effluent added. Addition of secondary treated sewage effluent resulted in a shift to nitrogen as the primary growth-limiting nutrient. Dilution with a low nutrient water linearly decreased the AGP. The control of phosphorus inputs into the impoundment could be used to reduce the eutrophication level. (Abstract-Author).

0128-d

Steyn, D. J., D. F. Toerien, and J. H. Visser. 1976. Eutrophication levels of some South African impoundments. IV. Vaal Dam. Water S.A. 2(2):53-57.

eutrophication, impoundment, wastewater, nutrients, nitrogen, phosphorus, AGP, algal assay, algae, Selenastrum capricornutum.

The Vaal Dam shows algal bioassay growth rates and algal growth potentials (AGP) as high as 1.6 d^{-1} and 146 mg/l^{-1} respectively using Selenastrum capricornutum as test alga. Addition of secondary treated sewage effluents to Vaal Dam water increased the AGP by between 7.4 and 11.2 mg/l^{-1} for every one percent (v/v) added. Nitrogen and phosphorus were the important algal grow-limiting nutrients in the impoundment. Plant nutrients adsorbed onto clay particles could be important in the eutrophication of the impoundment, indicating the importance of soil conservation techniques in eutrophication control. The significance of the impoundment and the paucity in knowledge of its physical, chemical and biological characteristics suggest that such studies should be undertaken as a matter of urgency. (Abstract-Author).

0129

Sturm, R. N., and A. G. Payne. 1973. Environmental testing of trisodium nitrilotriacetate: Bioassays for aquatic safety and algal stimulation, p. 403-424. In E. Glass [ed.], Bioassay techniques and environmental chemistry, Ann Arbor Sci., Mich.

toxicity, wastewater, phosphate, NTA, trisodium nitrilotriacetate, algal assay, bottle test, lake waters, iron, phosphorus, nitrogen, algae, Selenastrum capricornutum, Microcystis aeruginosa, Anabaena flos-aquae, Navicula seminulum, fish, diatoms.

While data for chronic toxicity studies have not yet been released, information is available from an extensive acute toxicity program which includes several species as well as three degrees of water hardness. The relative non-toxicity of trisodium NTA, even in very soft water where its effects would be the greatest, and the absence of evidence of accumulative toxicity suggest NTA to be compatible with the goal of environmental improvements. While this work is but a small part of the total NTA environmental safety test program, it does produce evidence that 1) NTA exerts no acute toxic effect on the fish or algae species tested at levels well above those anticipated in the environment, 2) Bluegills and fathead minnows exposed to high but sublethal concentrations for a period of 28 days do not exhibit abnormal gill pathology, a further indication of NTA's safety, and 3) NTA stimulatory effects on algae would not be anticipated on the basis of nitrogen contribution and were not found by application of the AAP Bottle Test to a variety of lake waters. (Summary-Author).

0130

Sugiki, A. 1977. Algal growth potential of tertiary effluents. Japan Sewage Works Agency. Dept. of Research and Development (unpublished).

eutrophication, wastewater, phosphorus, nitrogen, nutrients, algal assay, AGP, water quality, stimulation, algae, Chlorella sp., Selenastrum capricornutum, Chlamydomonas sp., Stigeoclonium tenue, lake waters.

Algal assays were used to determine the effects of the sewage effluents and removal of phosphorus from the effluents on algal growth rates. The AGP parameter was measured as the primary production rates within the water bodies. Water samples from lakes and creeks of Japan were assessed. The test algae used were _Chlorella_ sp. and _Selenastrum capricornutum_. Results and discussion are given for the AGP in natural waters and activated sludge - treated effluents and effluents from other listed sewage treatment processes. (Abstract-Leischman-EPA).

0131

Thomas, N. A., K. Hartwell, and W. E. Miller. 1975. Great Lakes nutrient assessment, p. 226-243. _In_ Proc. Biostim. Nutr. Assess. Workshop, 16-17 October 1973, U.S. Environmental Protection Agency, Corvallis, Oregon. EPA-660/3-75-034.

lake waters, nutrients, nitrogen, phosphorus, limiting nutrients, algal assay, bottle test, algae, _Selenastrum capricornutum_, phytoplankton, _Cladophora_.

A series of studies were conducted to assess the nutrient status of the Great Lakes. The first of the series was designed to determine the nutrient requirements of the green alga _Cladophora_, a problem alga in the Great Lakes. The studies were also to determine the availability of nutrients in the waters, especially those nutrients stimulating algal growth (nitrogen and phosphorus), and the effects of the addition of alum or iron salts to reduce phosphorus concentrations. The final portion of the series was to find an appropriate algal assay procedure for Great Lakes research, using the alga _Selenastrum capricornutum_ and natural phytoplankton populations. (Abstract-Leischman-EPA).

0132

Thomas, R. E., and R. L. Smith. 1975. Assessing treatment process efficiency with the algal assay test, p. 244-248. _In_ Proc. Biostim. Nutr. Assess. Workshop, 16-17 October 1973, U.S. Environmental Protection Agency, Corvallis, Oregon. EPA-660/3-75-034.

wastewater, nutrients, nitrogen, phosphorus, eutrophication, lake waters, algal assay, bottle test, algae, _Selenastrum capricornutum_.

The algal assay test was included in the evaluation of wastewater treatment processes for feedlot runoff and for municipal wastewater. The biomass productivity was directly related to the total nitrogen and/or phosphorus concentration in the product water from the treatment process. The results of these exploratory tests suggest that the algal assay test has promise for comparing the eutrophying potential of the effluents from a variety of wastewater treatment processes. This capability to measure eutrophying potential could be an important factor in determining cost effectiveness of treatment processes in many localities. (Summary-Author).

0133

Tison, D. L., and A. J. Lingg. 1977. Algal bacterial mutualism in a simulated aquatic environment. Annu. Meet. Am. Soc. Microbiol. 77(1):237.

organics, carbon, carbon dioxide, mutualism, glucose, stimulation, algae, Anacystis nidulans, Anabaena flos-aquae, Chlorella pyrenoidosa, Selenastrum capricornutum, algal assay.

The effects of increasing levels of organic enrichment in unialgal-bacterial cultures of non-limiting nutrient levels were determined. The closed system of an electrolytic respirometer was employed to follow the fate of added organic carbon. Glucose additions of 14, 64, and 128 mg C l^{-1} were made daily. Glucose additions contained 3 μCi D-glucose-UL-^{14}C. After six to ten days each system was assayed for carbon dioxide evolved to the atmosphere, inorganic carbon in solution, dissolved organic carbon, bacterial biomass carbon, and algal biomass carbon. Bacterial metabolism of added substrate and the resulting production of carbon dioxide and/or cofactors stimulated the growth of the cyanobacteria Anacystis nidulans and Anabaena flos-aquae and the green algae Chlorella pyrenoidosa and Selenastrum capricornutum. Autotrophic oxygen production provided a major portion of the oxygen required by the heterotrophic community. The association between the autotrophic cyanobacteria and green algae and the heterotrophic bacteria in this system was mutualistic. (Abstract Only-Author).

0134

Toerien, D. F. 1974. Half saturation constant for nitrogen limited growth of the green alga Selenastrum capricornutum. South Afr. J. Sci. 70(3):75-76.

algal assay, kinetics, limiting nutrients, nitrogen, continuous flow, algae, Selenastrum capricornutum Printz, eutrophication.

The report is a brief discussion of results obtained from a continuous flow algal assay conducted to determine the half-saturation content for nitrogen-limited growth of the test alga Selenastrum capricornutum Printz. Growth of the algae was found to be limited by nitrogen; the calculated kinetics value for Selenastrum capricornutum indicated a high affinity for nitrogen during the growth period. The author felt a need to conduct further experiments to test the limiting nutrient hypothesis and to aid in managing eutrophication problems. (Abstract-Leischman-EPA).

0135

Toerien, D. J., and C. H. Huang. 1973. Algal growth prediction using growth kinetic constants. Water Res. 7(11):1673-1681.

growth rates, kinetics, phosphorus, cultures, forecasting, time, eutrophication, biomass, nutrients, suspended solids, Selenastrum capricornutum Printz, algal assay, bottle test, algae.

The growth kinetics under conditions of phosphorus limitation of Selenastrum capricornutum Printz, a green alga specified for use in algal bioassays, were used to predict growth in batch cultures for varied specific conditions of time and phosphorus concentration. These predictions compared very well with actual batch culture growth studies. The predicted maximum cell concentration for two different levels of phosphorus lay within values obtained in different laboratories. The predicted maximum specific growth rates (μ_b) were either close to or just above actual laboratory data. The determination of growth kinetics thus allows accurate prediction of the growth of planktonic algae, a benefit in either algal bioassays or the solution of practical eutrophication problems. The growth kinetic constants of specific algae important in eutrophication problems need to be determined in order to utilize potentialities of prediction in the rational solution of these problems. (Abstract-Author).

0136

Toerien, D. F., and D. J. Steyn. 1974. The eutrophication levels of four South African impoundments. Verh. Int. Ver. Limnol. 19(3):1947-1956.

algal assay, eutrophication, wastewater, impoundment, AGP, limiting nutrients, nitrogen, phosphorus, nutrients, algae, Anabaena circinalis, Selenastrum capricornutum.

Algal assays were used to determine eutrophication levels of four South African dams, Hartbeespoort, Rietvlei, Roodeplaat, and Vaal. The algal growth potential (AGP) was measured for the test alga Selenastrum capricornutum. Identification of the limiting nutrients; indication of stimulation from additions of wastewater; and removal of nutrients by dilution with high quality water, were determined from the studies. The authors concluded that all four impoundments were eutrophic and algal growth was stimulated by the wastewater additions on a linear relationship. Nitrogen and phosphorus were the primary and secondary limiting nutrients; the AGP increased with increased concentrations of nitrogen. The removal of nutrients from the waters tested was thought to be the potential method to control eutrophication. (Abstract-Leischman-EPA).

0137

Toerien, D. F., K. L. Hyman, and M. J. Bruwer. 1974. Comparison of the algal bioassay responses of Selenastrum capricornutum and Microcystis aeruginosa. South Afr. J. Sci. 70(3):75.

algal assay, eutrophication, bottle test, AGP, impoundment, algae, Selenastrum capricornutum, Microcystis aeruginosa.

A brief discussion of results obtained from comparisons of growth rates (AGP) of two test algae, Selenastrum capricornutum and Microcystis aeruginosa, used in an algal assay for waters of Hartbeespoort Dam. Very significant correlation coefficients to growth were found between the two algae from the results. The authors found that the response of Selenastrum capricornutum can be related to the growth of Microcystis aeruginosa. Summarized regression analyses are given. (Abstract-Leischman-EPA).

0137-a

Toerien, D. F., K. L. Hyman, and M. J. Bruwer. 1975. A preliminary trophic status classification of some South African impoundments. Water S.A. 1(1):15-23.

algal assay, bottle test, limiting nutrients, nitrogen, phosphorus, impoundment, eutrophication, AGP, algae, *Selenastrum* *capricornutum*.

A preliminary trophic status classification of 98 major South African impoundments was constructed using algal bioassay procedures to determine the algal growth potential and to identify the growth limiting nutrients in each impoundment. The classification procedure embodied a technique whereby the growth potential of nitrogen fixing algae was accounted for. Although the classification is based on a single parameter, it appears to provide a useful guide to the trophic status of these impoundments. Approximately 50 percent of the impoundments were low in plant nutrients, 11 percent were heavily eutrophied and the rest were intermediate. Phosphorus was the most frequent algal growth limiting nutrient in non-eutrophied impoundments and nitrogen the most frequent in highly eutrophied ones. Because of the fixation of atmospheric nitrogen by certain blue-green algae, the control of nitrogen appears to have limited value in eutrophication control. Limiting the access of phosphorus to impoundments seems to offer a long-term solution to eutrophication problems. Urban-industrial development giving rise to nutrient high effluents, seems to be the major cause of eutrophication although the role of agriculture can not be negated. (Abstract-Author).

137-b

Toerien, D. F., D. J. Steyn, and S. I. Kohlmeyer. 1975. Nitrogen yield coefficient of the green alga, *Selenastrum* *capricornutum* Printz. South Afr. J. Sci. 71:217-218.

algal assay, growth kinetics, nitrogen, phosphorus, nutrients, limiting nutrients, algae, *Selenastrum* *capricornutum* Printz, impoundment, wastewater.

Water samples from various impoundments were tested to determine eutrophication levels. The samples were enriched with sewage effluent and varying concentrations of nitrogen. Yield coefficients for the test alga *Selenastrum* *capricornutum* were calculated for different concentrations of nitrogen and phosphorus; a growth curve was constructed. Tests conducted in the algal culture medium resulted in a predicted growth curve that was closely related to the curve from the actual test waters. The authors concluded that it was possible to predict growth of *Selenastrum* *capricornutum* under nitrogen limited conditions. (Abstract-Leischman-EPA).

0138

Toerien, D. F., C. H. Huang, J. Radimsky, E. A. Pearson, and J. Scherfig. 1971. Final report: Provisional algal assay procedures. Sanitary Engr. Research Lab. SERL Report, Berkeley, Calif., SERL Report No. 71-6. 211 p.

continuous flow, algal assay, nutrients, bottle test, lake waters, limiting nutrients, algae, Selenastrum capricornutum, rivers, wastewater.

Batch and continuous flow (chemostat) assays were investigated as part of a joint industry-government sponsored, multi-laboratory effort to develop a standardized algal assay procedure for nutrient level assessment. Assays were conducted with Selenastrum capricornutum as a standard assay organism.

Batch culture assays were found to have a lower level of precision than chemostat assays in the assessment of growth response as a function of nutrient concentration. The biomass parameter, maximum cell concentration, $\hat{X}$, of the batch assay generally responded to the nutrient concentration of the samples, however, the chemostat biomass parameter, steady state cell concentration, X_1, always was found to be proportional to the nutrient concentration of the samples.

The results of spiking tests with batch assays generally were inconclusive with respect to identification of the growth rate limiting nutrient whereas the results of spiking tests with chemostats indicated clearly the growth rate limiting nutrient. It is recommended that batch type algal assays be used only for crude screening or routine monitoring purposes and that the chemostat should be used for the quantitative assessment of the algal growth supporting properties of waters as well as for the development of kinetic descriptions for nuisance algae and the rate limiting nutrients of concern.

A kinetic description of Selenastrum capricornutum indicated a low half saturation constant, K_s, (the concentration of nutrient supporting one-half the maximum growth rate) of about 5 μg P/ℓ for phosphate phosphorus and a yield coefficient, Y, that varied as a function of growth rate. A theoretical model was proposed and evaluated which describes the varying yield coefficient (the result of "excess" uptake) as a function of the growth rate (mean cell residence time). The function was verified experimentally at a very high statistical confidence level. The significance of these findings and their application to the practical problem of eutrophication assessment is presented. (Abstract-Author).

0139

Trainor, F. R., and L. E. Shubert. 1973. Growth of Dictyosphaerium, Selenastrum, and Scenedesmus (Chlorophyceae) in a dilute algal medium. Phycologia 12(1/2):35-39.

algal assay, growth rates, rivers, bottle test, algae, Scenedesmus, Dictyosphaerium, Selenastrum, media.

Cultures were maintained in dilute laboratory media for weeks by frequent transfer of cells. Single strains of Scenedesmus and Dictyosphaerium were cultured in a medium with 28.5 mg/l total inorganic salts, with growth at the rate of 1 + doublings/day. Growth of a Selenastrum was compared in one experiment. Bristol's medium was also used for comparison.

Routinely, an actively growing inoculum was used, sufficient to give an initial cell concentration of approximately 2 X 10^5 cells/ml; this was adequate for colorimeter readings. Diurnal light of 3200 lux intensity was used throughout. Each day, after a determination of growth, the culture was transferred by dilution to the original cell number. Some cultures have been grown in this way for several weeks, and a uniform daily increment in growth is reported for 10-day experiments. Using this technique we record similar growth rates in sterile-filtered river water used as a medium. Stock cultures can be held in dilute media for several weeks, by first establishing a healthy population, and then maintaining the culture under minimal conditions, usually light at 160-215 lux. (Abstract-Author).

0140

U.S. Environmental Protection Agency. 1971. Algal Assay Procedure Bottle Test. U.S. Environmental Protection Agency, Corvallis, Oregon. 82 p.

algal assay, bottle test, eutrophication, nutrients, trace elements, growth rates, toxicity, pretreatment, inhibition, stimulation, PAAP, limiting nutrients, wastewater, chlorophyll, standing crop, temperature, light intensity, phosphorus, nitrogen, N:P ratios, experimental design, data interpretation, algae, Selenastrum capricornutum, Microcystis aeruginosa, Anabaena flos-aquae.

This document is the product of more than two years of intensive research at governmental, industrial, and university laboratories to develop a reliable and reproducible algal assay. The "Bottle Test" of the Provisional Algal Assay Procedure (PAAP), which was published by the Joint Industry/Government

Task Force on Eutrophication in February, 1969, served as the starting point for this effort. In improving and evaluating the test, each laboratory, of the participating group of nine, followed the same research plan using algal test species from a common source. This course of action identifies elements of the test that were faulty or difficult or questionable. As such specific problems were recognized, selected laboratories were assigned the task of probing them and developing sound adjustments. To do this they followed research plans developed jointly by all laboratories. As a result of this massive effort, the Algal Assay Procedure:Bottle Test was refined sufficiently to be used in connection with eutrophication and other algal production problems. (Condensed forward-Author).

0141

U.S. Environmental Protection Agency. 1974. Dillon Reservoir - Blue River Study, Colorado. U.S. Environmental Protection Agency, Denver, Colorado. S & A / TIB - 28. 35 p.

reservoirs, eutrophication, wastewater, nutrients, rivers, algal assay, molybdenum, growth rates, algae, Selenastrum capricornutum, phosphorus, bottle test.

Studies of the Dillon Reservoir - Blue River area involved algal assays to determine nutrient levels; the effects of nutrient loadings from wastewater discharge on algal productivity; and the possible effects, if nutrient loadings increased. The algal growth potential was measured for the alga Selenastrum capricornutum in laboratory tests. High levels of molybdenum were caused from mining activities; the algal assay was used to determine effects of molybdenum on the growth of Selenastrum capricornutum in the waters. (Abstract-Leischman-EPA).

0142

U.S. Environmental Protection Agency. 1974. Biostimulation characteristics of wastes and receiving waters of the Snake River basin. National Field Investigations Center - Denver, Colorado and U.S. Environmental Protection Agency, Seattle, Washington. 49 p.

reservoirs, rivers, water quality, wastewater, nutrients, stimulation, phosphorus, feedlot runoff, algae, Selenastrum capricornutum, algal assay.

Studies were designed to assess the characteristics of wastewater being discharged into the Snake River basin. Algal assays were used to determine the nutrient levels required to stimulate algal production; to provide information that would aid in the removal of nutrients from the waters; and to add further information pertaining to the development of discharge permits. Research was conducted in the laboratory and field. (Abstract-Leischman-EPA).

0143

U.S. Geological Survey. 1975. Bioassay: Algal growth potential (AGP), p. 289-293. In P. E. Greeson, T. A. Ehlke, G. A. Irwin, B. W. Lium, and K. V. Slack [eds.], Methods for collection and analysis of aquatic biological and microbiological samples, Ch. A4, Book 5, U.S. Geological Survey, Washington, D. C.

AGP, water quality, limiting nutrients, electronic particle counter, algal assay, pretreatment, algae, Selenastrum capricornutum.

The report is a modification of the US EPA 1971 Algal Assay procedure using the algal assay and the algal growth potential parameter. Methodology, apparatus, reagents, collection, analysis, calculations, appendix and references given. (Abstract-Leishman-EPA).

0144

Venkataraman, G. S., and K. V. Natarrajan. 1958. Molybdenum requirements of Selenastrum westii. Current Sci. 27(11):454-456.

molybdenum, growth rates, toxicity, trace elements, bottle test, algae, Selenastrum westii, Scenedesmus obliquus, algal assay.

The report is of a study conducted to determine the molybdenum requirements for the alga Selenastrum westii. The authors describe the methods followed in the experiment and the results, in terms of growth rates. Selenastrum westii was found to have a very low molybdenum requirement, as compared to the requirement for Scenedesmus obliquus. The level of molybdenum that was inhibitory to Selenastrum westii was also found to be less than the level for Scenedesmus obliquus. (Abstract-Leischman-EPA).

0144-a

Vyas, H. 1975. The effects of dyes on environment, p. 251-269. In Proc. Third Annual Poll. Control Conf. Water and Wastewater Equip. Manuf.

Assoc. Industry Solutions '75, Air-Water-Noise-Solid Waste, Ann Arbor Sci. Mich.

dyes, pollution, toxicity, heavy metals, wastewater, anaerobic, aerobic, inhibition, stimulation, algal assay, bottle test, algae, *Selenastrum capricornutum*, fish, *Pimephales promelas*.

An attempt has been made in this paper to present some of the environmentally related studies that ADMI has sponsored in the past. These studies represent only the thin edge of the wedge. There is much left to be done, however, we are very encouraged by the results obtained so far which seem to show that dyes, for the most part, do not present serious problems to the environment.

In our continuing quest for greater understanding of the role of dye in the ecosystem, ADMI is currently directing a study, partly funded by EPA, to investigate treatability of dying wastes by various biological and physico-chemical treatments. This work is being carried out at the North Carolina State University at Raleigh, and the University of North Carolina at Chapel Hill. As a part of this project, a survey of textile mill effluents will be carried out for selected heavy metal contents, TOC and benzidine. (Summary-Author).

0145

Walmsley, R. D., and P. J. Ashton. 1977. Algal growth potential and the succession of algae in a nitrogen-limited impoundment. South Afr. J. Sci. 73(5):151-152.

algal assay, bottle test, impoundment, AGP, nutrients, nitrogen, phosphorus, succession, limiting nutrients, algal blooms, algae, *Anabaena flos-aquae*, *Selenastrum capricornutum*, phytoplankton, *Anabaena circinalis*, *Volvox rousseletii*, *Microcystis aeruginosa* Kutzing, chlorophyll *a*, standing crop.

An algal assay was used to test waters from the Rietvlei Dam and to determine the potential of the waters to support algal growth. Two test species were used, *Selenastrum capricornutum* and *Anabaena flos-aquae*. AGP values were calculated; standing crop was estimated by analysis of chlorophyll *a*. Results showed that phosphorus was always abundant and available to algal growth; nitrogen was in limiting supply. The AGP values for the two test algae were found to relate to algal blooms and succession within the impoundment. The nitrogen supply was thought to be the governing factor of succession of the algae. Although the results yielded high AGP values, the authors did not conclude that the values meant the development of large algal blooms. The authors felt this indicated limitations involved with using assays to predict algal growth. (Abstract-Leischman-EPA).

0146

Walmsley, R. D., and D. F. Toerien. 1975. Algal bioassays and nitrogen fixation. South Afr. J. Sci. 7(10):313-314.

nitrogen fixation, algal assay, bottle test, impoundment, limiting nutrients, AGP, phosphorus, nutrients, algae, *Selenastrum capricornutum*, *Anabaena flos-aquae* (Tischer's-strain A37), eutrophication.

Studies were conducted in order to understand results from algal assays of test waters where nitrogen is the primary limiting nutrient and phosphorus the secondary limiting nutrient. Comparisons of two test algae, *Selenastrum capricornutum* a green, non-nitrogen fixing alga; and *Anabaena flos-aquae* a blue-green, nitrogen fixing alga, and the respective algal growth potential values are given from tests of waters of Rietvlei Dam. (Abstract-Leischman-EPA).

0146-a

Walmsley, R. D., and D. F. Toerien. 1977. The summer condition of three eastern Transvaal reservoirs and some considerations regarding the assessment of trophic status. J. Limnol. Soc. South Afr. 3(2):37-41.

algal assay, bottle test, nutrients, nitrogen, phosphorus, chlorophyll *a*, algae, *Selenastrum capricornutum* Printz.

Summer temperature, oxygen and light penetration profiles in the Da Gama, Klipkopje and Witklip Reservoirs are presented. Analysis of waters from different depths showed the presence of chemical stratification despite the absence of clear thermal stratification. An algal bioassay procedure also confirmed the presence of increasing quantities of available nutrients with depth in all three reservoirs. The significance of the results in assessing trophic status is discussed and it is concluded that for South African impoundments, an anaerobic hypolimnion is not exclusively associated with eutrophy. (Summary-Author).

0147

Walmsley, R. D., W. E. Scott, and D. F. Toerien. 1975. A laboratory evaluation of a dye for the control of algal growth. Water SA 1(2):90-92.

dyes, growth rates, algal assay, bottle test, chlorophyll *a*, light penetration, algae, *Selenastrum capricornutum*, *Anacystis nidulans*.

The principle of the use of a dye for the control of algal growth was investigated at the laboratory scale. The commercial dye employed was shown to have little effect on the growth rates of *Anacystis nidulans* and *Selenastrum capricornutum* in the laboratory at concentrations above the recommended doses. Experiments using 200 ℓ tanks also substantiated this lack of effect on mixed phytoplankton populations. It is suggested that a dye with absorption characteristics closer to those of algal pigments might be more effectual for algal growth control. (Abstract-Author).

0148

Walmsley, R. D., D. F. Toerien, and D. J. Steyn. 1978. An introduction to the limnology of Roodeplaat Dam. J. Limnol. Soc. South Afr. 4(1):35-52.

impoundment, eutrophication, nutrients, oxygen, AGP, nitrogen, phosphorus, chlorophyll *a*, phytoplankton, *Potomogeton*, *Microcystis*, *Microcystis aeruginosa*, toxicity, *Melosira granulata*, algae, *Selenastrum capricornutum*, algal assay, diatoms.

A two-year limnological survey between 1973 and 1975 showed that the Roodeplaat Dam is a warm monomictic impoundment which has stable thermal stratification during summer. Oxygen supersaturation of the surface waters and anaerobic conditions in the hypolimnion were encountered during summer. Water transparency was seasonal and related to existing phytoplankton populations and allocnthonously-introduced clay material. Chemical stratification during summer was prominent and appeared to be caused not only by mineralization processes in the hypolimnion, but also by dilution in the surface layers during summer flooding. Because of the morphometry of the impoundment and the fact that sewage effluents are discharged into the major inflow, considerable horizontal variation in most chemical and biological characteristics was found. On the basis of the nutrient content of the waters and the magnitude of the phytoplankton populations observed, Roodeplaat Dam may be classified as a highly eutrophic water-body. (Summary-Author).

0149

Walmsley, R. D., D. F. Toerien, and D. J. Steyn. 1978. Eutrophication of four Transvaal dams. Water SA 4(2):61-75.

rivers, impoundment, eutrophication, wastewater, nutrients, algal assay, AGP, algae, Selenastrum capricornutum.

A two year study of river waters in the catchment areas of the Buffelspoort, Hartbeespoort, Rietvlei and Roodeplaat Dams was conducted. Sources of eutrophying elements were detected by chemical analyses and algal bioassays. The degree of enrichment of each impoundment was compared by means of phosphorus surface loading rates. Treated sewage effluents which are discharged into the inflow waters of the Hartbeespoort, Rietvlei and Roodeplaat Dams are the main cause of their present enriched condition. (Abstract-Author).

0150

Walton, C. P., and G. F. Lee. 1972. A biological evaluation of the molybdenum blue method for orthophosphate analysis. Verh. Int. Ver. Limnol. 18(2):676-684.

molybdenum, orthophosphate, lake waters, algal assay, bottle test, phosphorus, limiting nutrients, algae, Selenastrum capricornutum.

A study has been conducted on the relationship between the results of the molybdenum blue method for measuring soluble orthophosphate in natural waters and the soluble phosphate concentrations present in natural waters which are readily available for algal growth under standardized laboratory culture techniques. The green alga Selenastrum capricornutum, was used as the test organism. The lake waters tested were fortified with the nutrients contained in the Provisional Algal Assay Procedure media such that the growth of the test organism would be limited by the amount of phosphorus available in the test water. It was found that there was no discrepancy between the amounts of growth of the test organism in the Lake Mendota water, sediment extracts, and algal detritus extracts predicted by the molybdenum blue soluble orthophosphate and the growth of the test organism in the test media with a corresponding amount of soluble orthophosphate added. (Summary-Author).

0150-a

Weiss, C. M. 1969. Batch culture tests: Effects of inoculum age and micronutrients on growth of Selenastrum in PAAP medium. Univ. North Carolina, Chapel Hill, N.C. Interim Report 1. FWPCA 16010 DQT.

algal assay, algae, Selenastrum, inoculum age, PAAP, media, growth rates, micronutrients, bottle test.

Experiments were designed and carried out to resolve the following questions:

a) To what extent does algal inoculum age affect viability and nutrient uptake on subculturing into PAAP medium.

b) Is it possible to maintain a stock algal culture in steady state conditions by withdrawing a constant volume of liquids from culture flasks, and replacing with an equivalent volume of fresh medium.

The overall objectve is to determine the optimum pre-culture conditions necessary to obtain a viable inoculum of Selenastrum with minimum nutrient content in the cells, for use in assaying lake waters.

This report deals with the first half of a series of experiments, involving studies on the effect of aging cultures for 5-21 days, and of maintaining a steady state culture, on subsequent viability and nutrient uptake, as determined by subculturing in different dilutions of the PAAP medium (October 1969 version). Good replication and a uniformity of trends in regard to the basic features of algal growth and nutrient uptake have confirmed previous results obtained by Weiss and other workers.

The above experiments were conducted with 50 ml liquid volumes in 125 ml culture flasks with an initial algal cell concentration of 10^3 cells/ml. (Modified Summary-Author).

0151

Weiss, C. M. 1970. Batch culture tests: Effects of inoculum age and micronutrients on growth of Selenastrum in PAAP medium. Univ. North Carolina, Chapel Hill, N.C. Interim Report 2. FWPCA 16010 DQT.

inoculum age, PAAP, media, growth rates, micronutrients, carbon, nitrate, bottle test, algal assay, algae, Selenastrum.

This report presents the results of experiments which replicate the experiments reported in Interim Report #1 in which the effect of age of culture on the growth of Selenastrum in PAAP medium was examined. Growth response was compared at 800 fc and 400 fc in 10 and 100% PAAP media with and without micronutrients. Detailed examination of the results of the second age of culture experiment is made and statistical comparisons of the two experiments is presented. The conclusions drawn indicate that age of culture between 7 and 21 days has no statistically significant effect on growth. No significant effect of light intensity between 800 and 400 fc on algal growth could be demonstrated but the higher light intensity did appear to produce significantly greater carbon production in 100% PAAP. The rate of NO_3-N uptake was considerably higher in the +m PAAP than in -m PAAP and thus appears to be dependent on the presence of micronutrients. The presence of micronutrients in PAAP medium is indicated as highly desirable since in all comparative experiments micronutrients enhanced the growth of algae, carbon synthesis and NO_3-N uptake. (Summary-Author).

0152

Weiss, C. M. 1970. Batch culture tests: Effects of inoculum age and micronutrients on growth of Selenastrum in PAAP medium. Univ. North Carolina, Chapel Hill, N.C. Interim Report 3. FWPCA 16010 DQT.

growth rates, inoculum age, media, bottle test, algal assay, algae, Selenastrum, nutrients.

Studies were conducted in two laboratories, University of Wisconsin and University of North Carolina, on the effects of inoculum age and media concentrations on specific growth rates of Selenastrum. A comparison of the two laboratory's conclusions is outlined in this report. Tables of specific growth rates and final yields are given. (Abstract-Leischman-EPA).

0153

Weiss, C. M. 1970. Effect of pH on precipitation and resolubilization of phosphate-phosphorus in algal cultures. Univ. North Carolina, Chapel Hill, N.C. Interim Report 4. FWPCA 16010 DQT.

pH, phosphorus, media, culture age, phosphate, precipitation, nutrients, resolubilization, algae, Selenastrum, algal assay, bottle test.

The report is of a study conducted to determine the effects of pH on the precipitation and resolubilization of phosphorus within algal cultures. Cultures of Selenastrum used in the tests, ranged from six to 34 days of age. Maximum removal of $PO_4{\equiv}P$ from the media was found to occur around day seven of incubation. It was found that adjustment of the pH by acidification did not greatly increase the $PO_4{\equiv}P$ concentration in the media, compared to samples untreated; however, the increase of $PO_4{\equiv}P$ in cultures older than 13-27 days was greater after acidification. The author concluded that in comparing samples that were acidified to samples unacidified, it was found that some increase in dissolved $PO_4{\equiv}P$ concentrations did occur following acidification in the majority of samples. (Abstract-Leischman-EPA).

0154

Weiss, C. M. 1970. Batch culture tests: Effects of inoculum size on growth of Selenastrum in PAAP medium. Univ. North Carolina, Chapel Hill, N.C. Interim Report 5. FWPCA 16010 DQT.

inoculum, growth rates, PAAP, media, carbon, phosphate, nitrate, bottle test, algal assay, algae, Selenastrum.

This report presents the results of an experiment designed to examine the effect of inoculum size on the growth of Selenastrum in +m and -m 100% PAAP media at 800 fc. Cultures were inoculated with 1,000, 10,000 and 50,000 cells/ml and measurements of algal numbers, algal carbon content, PO_4-P and NO_3-N concentrations were made after 5, 7, 9 and 14 days incubation. The results indicate that inoculum size has no effect on algal yield in either +m or -m PAAP after 14 days incubation. Higher yields are produced in the +m PAAP, however, than in -m. The PO_4-P uptake rate was found to increase with increasing inoculum size and was, in general, greater in +m PAAP than in -m. Although inoculum size had little effect on the NO_3-N uptake rate, the +m PAAP medium greatly enhanced the rate of NO_3-N utilization. The inoculum size had little effect on carbon synthesized in either +m or -m PAAP but there was a large difference in the amount of carbon synthesized by cells grown in the two media. (Summary-Author).

0155

Weiss, C. M. 1970. Factors affecting growth of Selenastrum in batch cultures: Removal of samples, surface-volume ratio of media, carbon limitation. Univ. North Carolina, Chapel Hill, N.C. Interim Report 6. FWPCA 16010 DQT.

algal assay, bottle test, growth rates, surface:volume ratios, carbon limitation, PAAP, algae, Selenastrum.

Three experiments were conducted to investigate the effects of various procedural aspects on the growth of Selenastrum in the Provisional Algal Assay Procedure. Experiment I contrasted the growth of Selenastrum in cultures from which samples were removed periodically with the growth in cultures from which no samples were taken. Experiment 2 was conducted to determine the effect of surface-volume ratio, as reflected by the various combinations of flask size and initial culture volume as well as the removal of different size samples, on the growth of Selenastrum. The effect of surface-volume ratio, in terms of the potential carbon limitation possibly imposed upon the cultures of low surface-volume ratio, was investigated in Experiment 3. In this experiment, differences in growth attributable to the use of aluminum foil vs. foam plastic plugs because of gas exchange limitations were also studied.

The results indicate that the surface-volume ratio of the culture exerts a profound influence on the growth of Selenastrum in 100% PAAP. The increased surface-volume ratio produced by the periodic removal of samples resulted in a higher algal yield. Likewise, variations in growth were observed in different flask sizes which reflected the differences in surface-volume ratio among the flasks.

It was found that in 10% PAAP neither surface-volume ratio nor the addition of double the normal amount of $NaHCO_3$ affected the total yield of algae indicating that carbon limitation did not occur in this medium.

Little difference in growth was noted between cultures grown with foil caps and those stoppered with foam plastic plugs. (Summary-Author).

0156

Weiss, C. M. 1970. Batch culture tests: The effects of surface volume ratio on growth of Selenastrum in PAAP media of varying strengths. Univ. North Carolina, Chapel Hill, N.C. Interim Report 7. FWQA 16010 DWT.

PAAP, media, surface:volume ratios, carbon, nutrients, algae, *Selenastrum*, bottle test, algal assay.

Cultures of *Selenastrum* were grown in a variety of strengths of PAAP medium under three different surface volume ratios. All other ambient conditions were held constant. Algal number and total carbon were used for indices of algal yield. A proportional relationship between yield and nutrient strength from 0 to 100 percent PAAP was attained after 21 days incubation in cultures with a surface volume ratio of 1.04. At lower surface volume ratios proportionality was evident only at reduced medium strengths. In cultures with a surface volume ratio of 0.22 the correspondence between yield and nutrient strength was maintained to no more than 40% of the October 1969 PAAP.

It was suggested that either a medium strength of 30 percent of the October 1969 PAAP or that a surface volume ratio of at least 1.0 be used in subsequent PAAP studies. A possible carbon limitation effect is suggested by the results of these experiments. (Abstract-Author).

0157

Weiss, C. M. 1970. Batch culture tests: The effects of the micronutrient history of algal stock cultures on subsequent growth in media with or without micronutrients. Univ. North Carolina, Chapel Hill, N.C. Interim Report 8. FWPCA 16010 DQT.

media, PAAP, micronutrients, phosphorus, nitrogen, carbon, phosphate, nitrate, growth rates, algae, *Selenastrum capricornutum*, bottle test, algal assay.

Stock cultures of *Selenastrum* which had been carried consistently in one type of medium (PAAP with or without micronutrients) were transferred to both media types. After 9 days incubation, cells from each of these cultures were also transferred to both -M and +M PAAP media. These cultures were incubated 14 days, at which time measurements were made of PO_4-P and NO_3-N uptake, organic carbon and the growth in cell number.

The results indicated that no relationship between the micronutrient history of the culture and the growth in cell number, the amount of carbon assimilation, PO_4-P or NO_3-N uptake. The growth in cell number, the amount of carbon assimilation and the NO_3-N uptake in the -M medium were, however, consistently lower than in the +M medium, regardless of the history of the stock algae. (Abstract-Author).

0158

Weiss, C. M. 1970. Batch culture tests: The exchange of phosphorus between algal cells and media as related to the age of culture. Univ. North Carolina, Chapel Hill, N.C. Interim Report 9. FWPCA 16010 DQT.

phosphorus, carbon, nitrogen, PAAP, bottle test, algal assay, media, nutrients, algae, *Selenastrum capricornutum*.

Selenastrum were cultured in 100% (October 1969) PAAP media for 35 days. At intervals throughout the incubation period samples were taken and analyzed for PO_4-P, NO_3-N and organic carbon content, as well as growth in cell number. At each sampling a portion of the sample was inoculated into 10% PAAP media. These cultures were analyzed at 14 days for growth in cell number and organic carbon content.

Of primary interest in this work was: (1) the phosphorus content of the algal cells (cultured in 100% PAAP) as related to the age of the algae, (2) and the effects of the algal phosphorus content on the growth of these cells when inoculated into fresh medium. The results indicated a relatively high level of phosphorus in the algal cells of the 100% PAAP cultures during the first three days of incubation. The level dropped sharply while the cells were in the log phase of growth, to a low at about 7 days incubation. The algal phosphorus content remained constant, thereafter. Cells of 3 to 35 days of age, when inoculated into 10% PAAP medium, showed no significant differences in growth, measured in terms of algal number and organic carbon at 14 days incubation. (Abstract-Author).

0159

Weiss, C. M. 1970. Influence of nitrilotriacetate (NTA) on eutrophication *in situ* and batch assays. Univ. North Carolina, Chapel Hill, N.C. Interim Report 11. FWQA 16010 DQT.

nitrilotriacetate, NTA, stimulation, carbon-14, algal assay, bottle test, wastewater, nitrogen, carbon, phosphorus, lake waters, NAAM, media, impoundment, algae, *Selenastrum capricornutum*, phytoplankton.

The potential of nitrilotriacetate (NTA) to act as an algal growth stimulant was evaluated by *in situ* carbon-14 productivity determinations and batch assay according to the PAAP procedure. The NTA in pure chemical form as well as following passage through secondary and tertiary sewage treatment was tested in comparison with nitrogen and carbon provided from other chemical sources in amounts approximately equivalent to that found in the NTA molecule. In addition to untreated controls, the lake waters and NAAM media (0.3 PAAP media of October 1969), one test media included a phosphorus addition to the lake waters.

The in situ and batch assays were made on three North Carolina impoundments over the period of July to October 1970. Each lake water was used for four test series, of the in situ and batch assays respectively. The in situ experiments to determine effects of NTA on productivity as determined by carbon-14 uptake were of four hour duration and the sets of light-dark bottles were hung at a depth which provided 50% of surface illumination. Batch assays used 0.45 μ membrane filtered water reseeded with Selenastrum capricornutum at 10^3 cells/ml. Growth responses in the various test media were followed by cell counts through 21 days incubation at 24°C and 400 f.c. on an oscillatory shaker at 110 oscillations/min. Dry weight of cell mass formed and carbon synthesized were also determined at the end of the 21 day incubation period. Changes in pH of the media were also followed through the growth period.

Evaluation of the in situ productivity results found that the presence of NTA either in pure form or following passage through some level of sewage treatment produced no change in productivity, carbon fixed ($mg/l\text{-}hr^{-1}$), that exceeded the 90% confidence limits of the untreated lake water controls. The only materials added to the lake water which resulted in productivity values that were outside the confidence limits of the controls were the Na and K carbonates and those on several tests resulted in productivity levels that were lower than the controls.

The batch assays of NTA as an algal growth stimulant essentially confirmed the results of the productivity tests. No medium containing NTA either in pure form or after passage through secondary or tertiary sewage treatment levels resulted in growth, by any parameter used, which exceeded that of the specific control in any significant manner. The phosphorus supplement used for comparative purposes in this test, even though used at 1/5 the concentration originally specified resulted, in nearly every assay series, in growth greater than the control, the nitrogen supplement or the NTA addition.

One interesting aspect of the productivity determinations was the finding that if the productivity of the lake controls were compared to the total phytoplankton count of the sample and total insulation for the period of exposure there appeared to be indicated an optimum cell concentration and insolation for the peak productivity values. (Summary-Author).

0160

Weiss, C. M. 1970. A statistical analysis of the effect on nitrilotriacetate (NTA) and other algal nutrients on productivity *in situ* and algal assay when tested in a randomized twelve block design. Univ. North Carolina, Chapel Hill, N.C. Interim Report 12. FWQA 16010 DQT.

lake waters, nutrients, wastewater, NTA, nitrilotriacetate, nitrogen, carbon, carbon-14, uptake, statistical methods, algal assay, bottle test, *Selenastrum capricornutum*, stimulation, algae.

From the results of the preceding statistical analysis, it would appear that the presence of nitrilotriacetate (NTA) on entering an aquatic environment through a sewage treatement plant could very well provide additional nitrogenous nutrients which would be available for the support of algal growth. It would appear that the sewage treatment process does break down the NTA molecule but in doing so apparently makes more readily available the nitrogen for cellular metabolism as compared to the pure chemical. The pure chemical would appear to have no significant effect on algal growth as tested in the batch assay procedure although it did appear to significantly stimulate productivity as determined by the uptake of $NaH^{14}CO_3$. (Conclusions-Author).

0161

Weiss, C. M. 1976. Evaluation of the algal assay procedure. U.S. Environmental Protection Agency, Corvallis, Oregon. Ecological Research Series EPA-600/3-76-064.

lake waters, algal assay, bottle test, impoundment, rivers, nutrients, pretreatment, water quality, chlorophyll *a*, limiting nutrients, algae, *Selenastrum capricornutum*.

Evaluation of the algal assay bottle test and its relationship to the trophic state or nutrient levels of surface waters was examined in 44 lakes, impoundments and rivers in North Carolina in 345 separate assay sets. Of particular concern was the evaluation of the significance of the pretreatment procedure, autoclaving or filtration, upon growth of the reseeded alga in relationship to the original water quality.

A limnological data profile was developed for each of the bodies of water sampled. A data processing procedure was used to establish the relationship between water quality data and algal cell density, chlorophyll a and productivity.

The algal assay procedure provided an indication of limiting nutrient, phosphorus, nitrogen or both. By clustering all samples of similar nutrient limitation a basic nitrogen, phosphorus relationship emerged. When the ratio of soluble inorganic nitrogen to total soluble phosphorus was greater than 13 the waters were phosphorus limited; when the ratio was in the range of 9-12 both nutrients were limiting; and when the ratio was below 8 nitrogen was limiting.

The quantity of algal biomass grown in autoclaved or filtered samples, without the addition of nutrient spikes provides an indication of the relative trophic or nutrient level of the particular body of water, the former a measure of total growth potential and the latter ambient growth potential. (Abstract-Author).

0162

Weiss, C. M. 1976. Field evaluation of the algal assay procedure on surface waters of North Carolina, p. 29-76. In E. J. Middlebrooks, D. H. Falkenborg, and T. E. Maloney [eds], Biostimulation and nutrient assessment, Ann Arbor Sci., Mich.

algal assay, nutrients, nitrogen, phosphorus, lake waters, rivers, pond water, impoundment, chlorophyll, pretreatment, growth rates, limiting nutrients, N:P ratios, algae, Selenastrum capricornutum.

To establish relationships between assays and existing trophic levels of water bodies, algal assays were made of 44 North Carolina water bodies. Cell density decreased with inorganic nitrogen increase; chlorophyll remained relatively constant; productivity reached a peak in middle concentration ranges. In the growing season soluble nitrogen showed an inverse relationship between cell number and nutrients. Phosphorus values varied but were higher in winter. Chlorophyll showed a positive relationship to cell density, productivity, and nitrogen. In the growing season there was a parallel increase in organic nitrogen and soluble phosphorus and increased productivity, cell density, and chlorophyll. Selenastrum capricornutum growth after autoclaving or filtering water samples indicated total and ambient growth potentials

respectively. Growth responses to added phosphorus or nitrogen compared to growth with original nutrient levels indicated nitrogen:phosphorus ranges, soluble nitrogen:phosphorus determinations following limitation of either or both nutrients indicated that the ratio can define which nutrient is limiting for a particular water body. The standard assay procedure should be modified to limit the determination to biomass grown in reseeded samples after autoclaving and filtration. Whether a sample is limited in growth potential by the relative quantity of phosphorus or nitrogen can be determined from the total soluble nitrogen:phosphorus ratio. (Abstract-Buchanan-Davidson-Wisconsin WRSIC).

0163

Weiss, C. M., and R. W. Helms. 1971. Interlaboratory precision tests: An eight laboratory evaluation of the Provisional Algal Assay Procedure Bottle Test. National Eutrophication Research Program. U.S. Environmental Protection Agency, Corvallis, Oregon. 70 p.

algal assay, bottle test, media, nutrients, lake waters, PAAP, chlorophyll, fluorescence, carbon, statistical methods, algae, Selenastrum capricornutum.

In order to establish the validity of an algal assay procedure for the determination of algal nutrient levels in surface waters a suitable protocol was designed and followed by eight laboratories. This group consisted of one government laboratory, four university laboratories and three industrial laboratories. The basic procedure was to evaluate by use of the "bottle" or batch test the precision and reproducibility of the growth response of one test organism, Selenastrum capricornutum, in four media varying nutrient strength. The media was originally defined for the PAAP test and modified slightly in subsequent evaluations. The test media of this experiment were all dilutions of the PAAP medium.

In addition to following the growth of the test alga in the four "mandatory" media, several of the participating laboratories examined growth response in the basic medium minus specific nutrient elements. The test protocol also permitted evaluation of growth controlling factors such as continuous shaking versus handswirling, comparison of the use of the hemacytometer versus Coulter Counter in determining cell numbers, and the levels of correlation between cell mass and cell number as determined by optical density, chlorophyll fluorescence and total algal carbon.

The unusual nature of this evaluation in the numbers of laboratories participating and magnitude of the test size has permitted the statistical analysis to be developed to the extent that the results will set new guidelines for future assays of this type. (Summary-Author).

0164

Williams, P. H. 1975. Response of the Spokane River diatom community to primary sewage effluent. M.S. thesis, East. Wash. State College, Cheney, Wash. 38 p.

wastewater, species diversity, species composition, phosphate, nitrogen, diatoms, ammonia, heavy metals, inhibition, pollution, organics, algae, Selenastrum capricornutum Printz, algal assay.

This study examined the effects of the primary sewage effluent from the City of Spokane, Washington on the diatom community in the Spokane River. Both natural and artificial substrates were used at each of three sampling stations, one above and two below the sewage outfall, to determine the impact of the effluent on species composition, species diversity, and autotrophic index. Water chemistry samples were taken concurrently with diatom collections and composited for use in determining the algal growth potential of the water.

A total of 52 species in 18 genera of diatoms were identified. The flora was dominated by Achnanthes spp. with this genus accounting for 63.6 percent of the cells observed. Nine of the ten most abundant diatom taxa showed statistically significant changes in abundance below the sewage discharge. In each of these ten species there was a significant correlation between their numbers and orthophosphate or ammonia nitrogen concentrations.

Mean autotrophic index values of 203 above and 1005 below the sewage outfall indicate the effluent promoted an increase in heterotrophic biomass.

Results from algal assays using Selenastrum capricornutum Printz as a test organism suggest that total algal growth above the discharge may have been limited by phosphorus concentrations while below the effluent heavy metal inhibition seemed to predominate.

In terms of individual species abundance, mean diversity indices, floristic similarities between sampling stations, and mean autotrophic indices, the diatom community at the last downstream station had begun to recover from the effects of organic pollution introduced via the sewage. (Abstract-Author).

0165

Won, W. D., L. H. DiSalvo, and N. G. James. 1976. Toxicity and mutagenicity of 2, 4, 6-trinitrotoluene and its microbial metabolites. Appl. Environ. Microbiol. 31(4):576-580.

TNT, toxicity, mutagenicity, metabolites, algal assay, bottle test, algae, _Selenastrum capricornutum_, copepods, oyster larvae.

TNT (2,4,6-trinitrotoluene) of explosive grade is highly toxic to the freshwater unicellular green alga _Selenastrum capricornutum_, and to the marine copepod _Tigriopus californicus_, and the marine oyster larva _Crassostrea gigas_. It is also mutagenic to _Salmonella typhimurium_. On the basis of mutagenic assays carried out with a set of histidine-requiring strains of the bacterium, TNT was detected as a frameshift mutagen that significantly accelerates the reversion rate of a frameshift tester, TA-98. In contrast, the major microbial metabolites of TNT appeared to be nontoxic and nonmutagenic. (Abstract-Author).

0166

Wright, J. Jr., F. A. Camp, and J. Cairn, Jr. 1974. Preliminary algal bioassays to determine nutrients limiting algal productivity in Mountain Lake, Virginia. ASB Bull. 21(2):92.

lake waters, nutrients, limiting nutrients, phosphate, nitrate, algal assay, bottle test, algae, _Selenastrum capricornutum_, nitrogen, phosphorus.

Mountain Lake, the only natural lake in the unglaciated portion of the Appalachian Mountains, is located at an altitude of 1200 m. (3970 ft) in an undeveloped section of Giles Country, Virginia. It has a length of 1.2 km. and a width of 0.4 km. with a surface area of about 100 acres. Proposed development of areas immediately surrounding the lake prompted the authors to conduct preliminary studies to evaluate the lake's algal growth potential by determining (1) what nutrients were presently limiting algal productivity in the lake, and (2) what concentrations of these nutrients were necessary to cause a statistically significant increase in algal growth. Chemical analyses conducted in the summer and fall of 1973 indicated relatively constant nitrate and phosphate concentrations throughout the lake. Only a trace of phosphate (as orthophosphate) was present while nitrate levels were approximately 0.3

ppm. Previous researchers had reported low levels of nitrate and phosphates throughout the year and characterized the lake as being oligotrophic. Assays were conducted in the summer, fall and winter of 1973, using the alga _Selenastrum_ _capricornutum_ as the assay organism. Nutrient spiking techniques were generally those suggested in the Environmental Protection Agency's Algal Assay Procedure Bottle Test. Phosphorus appeared to be the primary limiting nutrient, and nitrogen became limiting when phosphorus was added in excess. (Abstract Only-Author).

2.0 Keyword Index

adenosine triphosphate

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Chlamydomonas reinhardtii
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Chlorella
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- 0028
- 0028-a
- 0032
- 0050-a
- 0063
- 0089
- 0099
- 0130

Chlorella ellipsoidea
- 0083

Chlorella fusca
- 0004

Chlorella fusca (strain 211-8b)
- 0021

Chlorella homosphaera
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- 0015
- 0017
- 0027-f

Chlorella kesslerii
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Chlorella ovalis Butcher
- 0119-a

Chlorella pyrenoidosa
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- 0067

Chlorella pyrenoidosa
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Coelastrum proboscideum

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Cyanophyceae

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Dictyosphaerium pullchellum

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Euglena gracilis

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Fremyella diplosiphon

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Gloeotrichia

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Gloeotrichia ecinulata

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Gomphonema

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indigenous phytoplankton

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0074

indolebutyric acid

0050-a

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inhibition (continued)

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inoculum age

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inorganic carbon

0086-a
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0084
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kinetics

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kinetics (continued)

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0135

Kirchneriella lunaris

0004

Kirchneriella subsolitaria

0085-a

lake waters

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0013
0014
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0017-a
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0020-c
0022-a
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0027-a
0027-c
0027-d
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0075
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0113-a
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0119-a
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0126-a
0126-b
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leachates

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Lemna minor

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light intensity

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light penetration

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limiting nutrients

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0017-a
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limiting nutrients
(continued)

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0054
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0061
0068
0069
0070
0073
0074
0077
0080
0080-a
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0086
0091
0094
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0126-a
0126-b
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0128-c
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0137-a
0137-b
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0166

lipids

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luminescence

0006

MAAP

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manganese

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0084

mathematical model

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0090
0104-a
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maximum yield

0027-c

media

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0073
0085-a
0108
0119-a
0126-c
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0150-a
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0163

Melosira granulata

0148

membrane filtration

0025

mercury

0024
0027-b
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mesotrophic

0025

metabolites

0165

Michaelis-Menten equation

0008
0086
0102

Microcystis

0027-a
0060-b
0148

Microcystis aeruginosa

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0003-a
0003-b
0003-c
0003-d
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0018
0020-b
0027
0027-b
0038
0048
0052
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0093

Microcystis aeruginosa

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0095
0096
0097
0109
0123
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0137
0140
0148

Microcystis aeruginosa Kutzing

0145

micronutrients

0150-a
0151
0157

Minitest

0014
0016
0017
0028
0031

molybdenum

0041
0144
0150

morpholine

0037

mutagenicity

0165

mutualism

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NAAM

0159

Navicula

0050-a

Navicula pelliculosa

0003-a
0003-b
0003-c
0067

Navicula seminulum

0048
0095
0097
0129

nickel

0013

nitrate

0010
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0061
0124
0151
0154
0157
0166

nitrilotriacetate

0159
0160

nitrite

0028

nitrogen

0011
0012
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0020-b
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0022-a

nitrogen (continued)

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0027-c
0027-d
0027-e
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0031
0031-a
0036
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0054
0055
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0059-a
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0063
0068
0070
0071
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0075
0076
0077
0080
0080-a
0081
0085-a
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0105
0108-a
0109
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0113-a
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0126-a
0126-b

nitrogen (continued)

0128
0128-a
0128-b
0128-c
0128-d
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0130
0131
0132
0134
0136
0137-b
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0145
0146-a
0148
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0164
0166

nitrogen fixation

0052
0146

nitrogen oxide

0075

nitroglycerine

0003-c

Nitzschia

0067
0118

Nitzschia palea

0126-d

Nostoc

0007

Nostoc commune

0089

Nostac linokia

0067

Nostoc muscorum

0061

N:P ratios

0003-e
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0012
0016
0016-a
0017-a
0022-a
0029-a
0054
0060-b
0077
0085-a
0140
0162

NTA

0082
0129
0159
0160

nuclear power

0037
0039

nutrients

0008-a
0009
0016-a
0020
0020-a
0020-b
0020-c
0022-a
0027

nutrients (continued)

0027-a
0027-c
0027-e
0028-a
0029
0030-b
0031-a
0050
0052
0059-a
0070
0073
0074
0075
0076
0079
0085
0085-a
0094
0098
0101
0102
0103
0104
0108-a
0111
0113-a
0114
0115
0116
0119-a
0120
0121
0122
0124
0125
0126
0126-a
0128-a
0128-b
0128-c
0128-d
0130
0131
0132
0135
0136
0137-b
0138
0140

nutrients (continued)

0141
0142
0145
0146
0146-a
0148
0149
0152
0153
0156
0158
0160
0161
0162
0163
0166

oil

0104-a
0104-b
0106

oil shale

0001-a

Oocystis marssonii

0118

Oocystis submarina var. *variabilis*

0030
0030-b

organic fractions

0072

organics

0018
0088
0109
0110
0125
0133
0164

organochlorine compounds

0001

orthophosphate

0150

Oscillatoria

0027-b

Oscillatoria agardii

0015

Oscillatoria geminata

0038

Oscillatoria redekii

0091

Oscillatoria tenius

0038

Oscillatoria Vaucher

0119-a

oxygen

0049
0148

oxygen electrode

0050

oyster larvae

0165

^{32}p

0008

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0108
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0150-a
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0157
0158
0163

PCB

0053

Pediastrum

0066

pH

0005
0041
0083
0086-a
0153

Phaeodactylum tricornutum

0015
0035
0053
0054
0055
0060

phenol

0104-b
0106
0107

phosphate

0007
0008
0008-a
0010
0023

phosphate (continued)

0026
0028
0030-b
0033
0037
0047
0056
0068
0083
0086
0096
0098-a
0113-a
0124
0129
0153
0154
0157
0164
0166

phosphorus

0003-d
0007
0011
0016-a
0019
0020
0020-a
0020-c
0022-a
0026
0027
0027-a
0027-c
0027-d
0027-e
0027-f
0028
0028-a
0029-a
0030
0030-b
0031
0031-a
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phosphorus (continued)

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0044
0045
0046
0051
0053
0054
0055
0057
0059-a
0060-b
0061
0062
0063
0066-a
0070
0071
0072
0073
0074
0075
0076
0077
0080
0080-a
0081
0084
0085-a
0086
0090
0091
0094
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0098
0099
0100
0101
0108-a
0109
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phosphorus (continued)

0126-a
0126-b
0128-a
0128-b
0128-c
0128-d
0129
0130
0131
0132
0135
0136
0137-a
0137-b
0140
0141
0142
0145
0146
0146-a
0148
0150
0153
0157
0158
0159
0162
0166

photohydrogen

0004

photosynthetic rates

0086-a

phytoplankton

0001-a
0003-b
0006
0008
0019
0022
0026
0027-a
0027-c
0027-d
0027-e

phytoplankton (continued)

0030
0034
0038
0045
0060-b
0066
0089
0091
0092
0095
0099
0112
0113
0119
0125
0126
0126-b
0131
0145
0148
0159

Pimephales promelas

0013-a
0065-a
0144-a

Poisson distribution

0003

pollution

0027-b
0027-e
0027-f
0032
0050
0052
0088
0104
0119
0119-a
0121
0127
0144-a
0164

pond water

0025
0162

Potomogeton

0148

precipitation

0153

pretreatment

0025
0051
0079
0111
0140
0143
0161
0162

protozoa

0001-b

Raphidiopsis curvata

0038

RDX

0003-a

refinery

0104-a
0106

reservoirs

0005
0022
0039
0046
0100
0101
0124
0125
0126

reservoirs (continued)

0141
0142

resolubilization

0153

restoration

0065

Rhizoclonium

0027-d

rivers

0002
0009
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0020-a
0020-b
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0027-d
0027-e
0034
0036
0043
0044-a
0045
0054
0059
0059-a
0076
0077
0079
0102
0114
0119
0119-a
0122
0125
0126-a
0126-b
0138
0139
0141
0142
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0162

Salmo gairdneri

0037

salt loading

0001-a

scenedesmine

0050-a

Scenedesmus

0004
0028
0050-a
0083
0139

Scenedesmus obliquus

0004
0087
0144

Scenedesmus obtusiusculus

0083

Scenedesmus quadricaruda

0001-a
0014
0017
0027-f
0029
0030
0030-b
0032
0041
0058
0060-a
0067
0089
0117
0118
0127

Scenedesmus quadricauda
Turpin em. Chod.

0028-a

sediments

0026
0034
0074
0098
0125

Selenastrum

0004
0027-a
0028
0029-a
0050-a
0066
0124
0139
0150-a
0151
0152
0153
0154
0155
0156

Selenastrum 235 (UTEX)

0126-c

Selenastrum 747 (UTEX)

0126-c

Selenastrum bibraianum

0098-b
0098-c

Selenastrum bibraianum Reinsch 324 (UTEX)

0126-c

Selenastrum capricornutum

0001-a
0002
0003
0003-a
0003-b
0003-c
0003-d

Selenastrum capricornutum
(continued)

0005
0006
0007
0008
0009
0009-a
0010
0011
0012
0013
0013-a
0013-b
0014
0015
0016
0016-a
0017
0017-a
0018
0019
0020
0020-a
0020-b
0020-c
0022
0022-a
0024
0025
0026
0027
0027-b
0027-c
0027-d
0027-e
0027-f
0029
0030
0030-a
0030-b
0031
0031-a
0032
0033
0034
0035
0036
0037
0038
0039
0040

Selenastrum capricornutum
(continued)

0041
0043
0044
0044-a
0045
0046
0048
0049
0050
0051
0052
0052-a
0053
0054
0055
0056
0057
0058
0060
0060-a
0060-b
0061
0062
0063
0064
0065
0065-a
0066-a
0067
0068
0069
0070
0072
0073
0074
0076
0077
0078
0080
0080-a
0081
0082
0083
0084
0085
0086
0086-a
0087
0090
0091

Selenastrum capricornutum
(continued)

0093
0094
0095
0096
0097
0098
0098-a
0099
0100
0101
0102
0103
0104
0104-a
0104-b
0105
0106
0107
0108
0108-a
0109
0110
0111
0113
0114
0117
0118
0119
0119-a
0120
0121
0123
0125
0126-a
0126-b
0126-d
0127
0128
0128-a
0128-c
0128-d
0129
0130
0131
0132
0133
0136
0137
0137-a
0138

Selenastrum capricornutum
(continued)

0140
0141
0142
0143
0144-a
0145
0146
0147
0148
0149
0150
0157
0158
0159
0160
0161
0162
0163
0165
0166

Selenastrum capricornutum Printz

0001
0008-a
0023
0028-a
0047
0059
0059-a
0071
0079
0112
0113-a
0115
0116
0126
0128-b
0134
0135
0137-b
0146-a
0164

Selenastrum capricornutum
Printz CHL 1 (NIWR)

0122

Selenastrum capricornutum
Printz 1648 (UTEX)

0126-c

Selenastrum capricornutum
ATCC #22662

0001-b

Selenastrum gracile

0042
0075
0089

Selenastrum gracile Reinsch

0003-e

Selenastrum gracile
Reinsch B 325 (UTEX)

0126-c

Selenastrum gracile
(Strain 278-2)

0021

Selenastrum minutum

0102-a

Selenastrum minutum (Näg.)
Collins 326 (UTEX)

0126-c

Selenastrum westii

0085-a
0144

Selenastrum westii G.M.
Smith CHL 17 (NIWR)

0122

silver

0027-b

Skeletonema costatum

0053
0054
0119-a

sodium chromate

0037

sodium citrate

0093

soil extraction

0003-e

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